UNIVERSITY OF OREGON

Biological Safety Manual

ENVIRONMENTAL HEALTH & SAFETY

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I. Biological Safety Program

1.1 Purpose

The University of Oregon's Biological Safety Program facilitates safe research involving recombinant or synthetic nucleic acid molecules (rsNA) and biohazardous materials. The Program seeks to fulfill this goal by providing support to the Institutional Biosafety Committee (IBC), managing the Bloodborne Pathogen Exposure Control Plan (ECP), and consulting on exposure assessments for the Occupational Health Program. This manual outlines appropriate practices, university policies and regulatory requirements for working safely with biological materials.

The University of Oregon is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing rsNA is necessary in many university research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules ("NIH Guidelines")* and other applicable federal, state, and local regulations, and incorporates best practices as outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

1.2 Scope

The University of Oregon Biological Safety Manual is applicable to all laboratory, research, teaching, service, and support activities that may involve exposure to biohazards. Biohazards are microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants. Often referred to as "infectious agents," examples include bacteria, bacterial toxins, viruses, fungi, rickettsia, prions, protozoans, parasites, genetically-modified organisms, or rsNA molecules. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin. The Biological Safety Program applies to all clinical, laboratory, research, service, and support activities the University sponsors or participates in.

1.3 Organization and Responsibilities

The Principal Investigator (PI) is directly and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying the recommendations in this manual. However, safety is a shared responsibility among all of the laboratory staff. Many resources exist to assist the PI with these responsibilities, including the Institutional Biosafety Committee (IBC) and the Environmental Health and Safety (EHS) department.

The University of Oregon

The president of the University of Oregon is ultimately responsible for all environmental health and safety issues at the institution. This responsibility is exercised through the normal chain of authority within the university by delegating the charge for ensuring safe work

practices and adherence to established policies and guidelines to the provost, vice-presidents, deans, directors, department chairs, PIs, supervisors and, ultimately, each employee (UO Safety Policy IV.05.01).

Environmental Health & Safety/ Biological Safety Officer (BSO)

- Assist departments in providing training and guidance for implementation of this policy.
- Develop, implement, and maintain a comprehensive biosafety program at the University of Oregon, including policies and procedures regarding biosafety principles and practices;
- Consult with UO Principal Investigators regarding mitigation of biological hazards, methods for compliance with applicable regulations, and biological waste disposal;
- Administer the Institutional Biosafety Committee;
- Track and coordinate annual inspections of biological laboratories and certification of biosafety cabinets on campus;
- Investigate accidents involving infectious agents and assist with corrective actions;
- Develop and provide training programs related to biosafety.

The Institutional Biosafety Committee

- Review recombinant and synthetic nucleic acid research conducted at or sponsored by the University for compliance with the NIH Guidelines, and approve those research projects that are found to conform with the NIH Guidelines;
- Review non-rsNA projects as defined in the IBC charter;
- Notify the PI of the results of the IBC's review and approval;
- Report any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illness to the appropriate Institutional official and to the NIH Office of Science Policy (OSP) as required;
- Follow the guidelines for membership and meetings as defined by NIH.

Principal Investigators

- Conduct the primary risk assessment of their experiments;
- Ensure the safe operation of their laboratory;
- Train laboratory personnel in safe work practices;
- Comply with all applicable state and federal regulations and guidelines;
- Register the following experiments with the IBC or EHS, as required:
  - recombinant and synthetic nucleic acid activities;
  - work with infectious agents;
  - experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, human cell lines, and certain body fluids;

  - animal and plant pathogens
- Report all spills, accidents, injuries, and potential exposures to the BSO

Laboratory Personnel

- Comply with safety recommendations for the work being performed;
- Report to their physician any concerns about risks and hazards of their laboratory work, particularly if they are pregnant or immunocompromised;
- Report all accidents, spills, or injuries to the PI.

II. Registration of Research

2.1 Review and Approval of Experiments

Principal investigators are responsible for registering with UO EHS BSO any experiments involving human materials, non-human primate materials, Risk Group 1,2 or 3 organisms, select agents and toxins, and recombinant or synthetic nucleic acids (rsNA), including those exempt from the *NIH Guidelines*. The biosafety office audits all laboratories where Biosafety level 2 (BSL2) containment is required, and all Biosafety Level 1 (BSL1) laboratories that are subject to the *NIH Guidelines*. The IBC, which oversees rsNA research at the University of Oregon, or the BSO will review and approve the registration. **Note: There are no BSL3 or BSL4 laboratories at the University.**

2.2 Experiments Covered by the *NIH Guidelines*

Many experiments involving rsNA require registration and approval by the IBC *PRIOR* to work may be initiated. Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4 or Restricted Agents as host-vector systems.
- cloning DNA from Risk Group 2, 3, 4 or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems
- infectious virus, or defective virus in the presence of helper virus in tissue culture systems
- whole plants or animals
- more than 10 liters of culture in a single vessel

Experiments that must be registered at the time of initiation include those that involve:

- the formation of rsNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture
- rsNA-modified whole plants, and/or rsNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the *NIH Guidelines*

- generation of transgenic rodents that require BSL1 containment.

2.3 Experiments Exempt from the *NIH Guidelines*

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. The BSO will review the registration and confirm that the work is classified correctly according to the *NIH Guidelines*. Exempt experiments are those that:

- Use synthetic nucleic acids that can neither replicate nor generate nucleic acids capable of replicating in any living cell; are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body weight.
- use rsNA molecules that are not in organisms or viruses
- consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent
- consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means
- consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species)
- consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent
- do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment
- contain less than one-half of any eukaryotic viral genome propagated in cell culture.
- use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host-vector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts
- involve the purchase or transfer of transgenic rodents for experiments that require BSL1 containment.

2.4 Human Blood, Unfixed Tissue, and Cell Culture

Please refer to the *Bloodborne Pathogens Exposure Control Plan* for detailed information on handling human-source material. Work with human-source material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR, Part 1910.1030) and comparable Oregon State regulations (OAR 437, Division 2, Subdivision Z). Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical

material should be presumed infectious and handled using BSL2 work practices. This concept is called Universal Precautions. Investigators are responsible for notifying EHS of their use of human materials so training and immunization can be provided as required by OSHA.

2.5 Biological Select Agents and Toxins

Biological Select Agents and Toxins (BSAT) are microorganisms and toxins that have potential for misuse by terrorists. The Federal Select Agent Program regulates the possession, use and transfer of select agents. Please contact the BSO immediately if you currently possess or plan to acquire any of these agents. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact the BSO, or visit the CDC's Select Agent website, www.selectagents.gov, which provides BSAT program information.

2.6 Non-Human Primate (NHP) Materials

Non-human primates and their tissues pose special zoonotic risks as many of their diseases are often transmissible to humans and can be a serious health hazard. Although there are a number of NHP viruses that can cause disease in humans, monkeys of the genus Macaca, or their unfixed tissues, can carry the virus *Macacine herpesvirus 1* (other terms used: Herpes B-virus, Herpesvirus simiae, or simply B-virus). B-virus is frequently carried by Rhesus, Japanese and Cynomolgus macaques, as well as other macaques. It can cause fatal encephalitis in humans.

Work with any NHP primary cell cultures or unfixed tissues must be registered with the IBC, and lab personnel must be trained in the safety procedures required for handling NHP material *PRIOR* to beginning the research. Sharps use with these materials should be eliminated or restricted.

III. Classification of Potentially Infectious Agents

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by federal and state regulations and guidelines. Many granting agencies require that grant recipients certify that they adhere to both the guidelines and the regulations.

The *NIH Guidelines* classifies pathogenic agents into one of four risk groups according to specific criteria.

3.1 Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	*NIH Guidelines Definition*	*World Health Organization Definition*
Risk Group 1	Agents not associated	*No or low individual and community risk*

	with disease in healthy adult humans.	A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.	*Moderate individual risk; low community risk* A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to lab workers, the community, livestock or the environment. Lab exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.	*High individual risk; low community risk* A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.	*High individual and community risk* A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

3.2 Microorganisms Capable of Causing Infection in Humans

Investigators must register work with human materials and non-recombinant work involving a Risk Group 2 and 3 with the BSO. Research using Risk Group 3 and/or 4 is not allowed on the UO campus. Contact the BSO for assistance in conducting a risk assessment and establishing proper containment and work practices.

3.4 Genetically Engineered Microorganisms

Following receipt of the completed new research registration form by the BSO, the laboratory will be surveyed to ascertain that it meets the containment requirements listed in the *NIH Guidelines* for the agent(s) being studied. If the lab meets the requirements, the work will be presented to the committee for review and approved or disapproved by the IBC.

Work with all genetically engineered organisms must comply with the *NIH Guidelines*. These guidelines classify experiments into four levels of containment, based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. It is University of Oregon policy that all laboratories follow these guidelines.

IV. Biosafety Containment Levels

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention

(CDC) publish guidelines for work with infectious microorganisms. The publication, entitled *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL1), BSL2, BSL3 and BSL4. The four biosafety levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. All of these factors, including the organism's Risk Group, are considered when assigning a biosafety level to work.

Microbiological work at the University of Oregon is conducted at BSL1 or BSL2 containment. There are no BSL3 or BSL4 laboratories at the University.

4.1 Summary of Biosafety Levels

Below is a summary of each biosafety level; detailed criteria for each level are described in Section IV of *BMBL.*

Biosafety Level 1 (BSL1)

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2 (BSL2)

Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Biosafety Level 2 builds upon BSL1. BSL2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

Biosafety Level 3 (BSL3)

Applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL3 laboratory has special engineering and design features. **The University of Oregon does not currently have a laboratory facility that meets BSL3 requirements.**

Biosafety Level 4 (BSL4)

Required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory**. The University of Oregon is not equipped for BSL4 work.**

4.2 Summary of Biosafety Level Recommendations

BSL 1

Agents: Not known to consistently cause disease in health adults.

Practices: Standard Microbiological Practices.

Primary Barriers & Safety Equipment: None required.

Secondary Barriers (Facilities): Laboratory bench and sink required.

BSL 2

Agents: Associated with human disease. Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.

Practices: BSL1 practices plus:

- Limited access
- Biohazard warning signs
- Restrict sharps use
- Biosafety manual defining any needed waste decontamination or medical surveillance

Primary Barriers & Safety Equipment: Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols. PPE: laboratory coats, gloves, face protection as needed.

Secondary Barriers (Facilities): BSL1 plus autoclave available.

BSL 3

Agents: Indigenous or exotic agents with potential for aerosol transmission. Disease may have serious or lethal consequences.

Practices: BSL2 practices plus:

- Controlled access
- Decontamination of all waste
- Decontamination of laboratory clothing before laundering

Primary Barriers & Safety Equipment: Class I or II BSCs or other physical containment devices used for all work. PPE: protective laboratory clothing, gloves, respiratory protection as needed

Secondary Barriers (Facilities): BSL2 plus:

- Physical separation from access corridors
- Self-closing, double-door access
- Exhaust air not recirculated
- Negative airflow into laboratory

BSL 4

Agents: Dangerous/exotic agents posing high risk of life- threatening disease. Transmission by aerosol route or unknown method of transmission.

Practices: BSL3 practices plus:

- Clothing change before entering
- Shower on exit
- All material decontaminated on exit from facility

Primary Barriers & Safety Equipment: All procedures conducted in Class III BSCs; or work performed in Class I or II BSCs in combination with full-body, air- supplied, positive pressure personnel suit.

Secondary Barriers (Facilities): BSL3 plus:

- Separate building or isolated zone
- Dedicated supply and exhaust, vacuum, and decontamination systems
- Others as outlined in BMBL

4.3 Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels (ABSL) 1, 2, 3, and 4, and

provide increasing levels of protection to personnel and the environment. **The University of Oregon conducts work at ABSL1 and ABSL2 only.**

One additional biosafety level, designated BSL3-Agriculture (or BSL3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL3-Ag facilities and USDA High Consequence Pathogens can be found in *BMBL*.

4.4 Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL2 are consistent with the OSHA standard, *"Occupational Exposure to Bloodborne Pathogens."* This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute.

BSL2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as Class II BSCs should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

V. Emergency Procedures

5.1 Biological Spills

A spill kit should be kept in each laboratory where work with microorganisms is conducted.

At a minimum, spill kits should contain concentrated disinfectant (such as chlorine bleach which should be freshly diluted prior to use), absorbent material, household rubber gloves, plastic waste bags, sharps container, and forceps to pick up broken glass.

5.2 Spill Clean-Up Protocol

Step	Procedure
1.	Any potentially contaminated clothing must be removed and placed in a biohazard waste bag. If the spill is inside a BSC, the BSC must remain running. If the spill is outside the BSC, notify room occupants to leave and allow 30 minutes for aerosols to settle before initiating cleanup.
2.	Hands and any contaminated skin must be washed thoroughly with soap and water.
3.	Put on appropriate PPE (at a minimum: disposable gloves, eye protection and a lab coat). Obtain the spill kit. Staff not needed for spill clean-up must be cautioned to stay away from the spill area. Signs may be posted if necessary.
4.	Any sharp contaminated objects must be removed from the spill area using mechanical means, never with hands.
5.	After all sharps are removed, cover the spill with absorbent materials such as paper towels.
6.	Use an appropriate disinfectant for the organisms present. If human blood or materials are involved in the spill, use **freshly diluted 10% bleach**. Disinfectant must be poured carefully around the edges of the spill, working from the outside of the spill toward the center. Allow 20 minutes contact time. Wet paper towels can be gathered using tongs and placed in plastic bag. ▪ If the spill is inside a centrifuge, the rotor and its contents should be moved to a BSC, if possible. ▪ If the spill is inside a BSC, the spill tray underneath the work area and the trough below the air intake grill must be cleaned as well. These are likely to be contaminated when the spill is large. **Note:** Alcohol is not recommended as a disinfectant for large spills.
7.	After initial clean-up, the affected area must again be saturated with disinfectant such as freshly diluted 10% bleach solution and left to soak for at least 20 minutes (adequate contact time is important to ensure complete decontamination).
8.	Disinfectant can be absorbed with paper towels. A final wipe-down should be done with clean paper towels soaked with disinfectant. Laboratory personnel should be sure to disinfect any equipment, walls or other areas likely to have been splashed by the spill. Be mindful of possible contamination of your shoes.
9.	All contaminated solid waste must be disposed of properly in the biohazard waste box.
10.	Hands must be washed thoroughly with soap and water. If the spill is inside a BSC, the cabinet should be left running for at least 10 minutes before resuming use.
11.	Report the incident to Safety & Risk Services. Any incident of a spill or exposure

	involving rsNA needs to be reported to Biosafety Officer. The NIH requires immediate reporting in the event of a significant research-related accident or problem.

5.3 Procedures for Cleanup of Human Blood

Only personnel enrolled in the UO Bloodborne Pathogens Program and appropriately trained may clean up blood spills. Follow the instructions outlined above, using a detergent solution (examples ALPHA HP at 1:64 ratio) for the initial cleanup and a freshly prepared 10% bleach solution for second round of disinfection.

5.4 Injuries Involving Biological Materials

For severe injuries:

- Call 911 for assistance and transportation to the nearest emergency room.
- Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
- Report accident to the PI and Environmental Health & Safety.
- Complete and submit Workplace Injury Form to Safety & Risk Services.

For splash to the eye:

- Immediately flush the eye in the eyewash station for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye.
- Students should contact the University Health Center (UHC) to obtain care. If UHC is closed, go to the nearest emergency clinic.
- Non-students should report to the nearest emergency clinic.
- Notify the PI and EHS, seek additional medical assistance if necessary.
- Complete and submit Workplace Injury Form to Safety & Risk Services.

For contamination of the hands or body:

- Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, flush the area for 15 minutes.
- Students should contact the UHC at to obtain care. If UHC is closed, go to the nearest emergency clinic.
- Non-students should report to the nearest emergency clinic.
- Notify the PI and to EHS, seek additional medical assistance if necessary.
- Complete and submit Workplace Injury Form to Safety & Risk Services.

Immediate evaluation by a medical professional is especially important after exposures to human blood. CDC recommends starting anti-retroviral drugs within two hours for significant exposures. The necessary appropriate prophylactic treatment can be started as soon as possible. Any relevant safety information about the pathogen should be brought with the patient. Referrals may be made to the workers' compensation medical provider for University of Oregon. The incident must be reported to EHS as soon as possible.

5.5 Fires in Biological Laboratories

Life safety is the highest priority. In case of fire, without placing yourself in danger, put biological materials in secure location, such as incubator or freezer. **Evacuate and Notify**. Activate the building fire alarm and leave the building at once. Call the fire department from a safe location. Meet the fire department outside and direct them to the fire. Any individual who receives an exposure or potential exposure will be given a medical consultation and advised of available treatments.

VI. Medical Surveillance

The Occupational Health Program provides medical surveillance for all personnel who are exposed to identified or regulated risks and for personnel with animal contact.

Workplace exposure to human blood and other potentially infectious materials (OPIM), as defined by the OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030), requires medical surveillance and annual Bloodborne Pathogens Training. University of Oregon has a written Bloodborne Pathogen Exposure Control Plan, available on the EHS website.

6.1 Vaccinations

UO Employee personnel working with human blood or OPIM must be offered the Hepatitis B vaccination at no cost to them. Whether or not the employee wishes to be vaccinated, a Hepatitis B Immunization/Declination Form must be completed the first time an employee receives Bloodborne Pathogens training as an UO employee. A copy of the form will be maintained with EHS. Personnel choosing to receive the vaccine will receive instructions from EHS for vaccination at the University Health Center, and the department's index # will be billed. Employees who initially decline the vaccine can change their mind at a later date and have the right to request the vaccine.

The tetanus vaccine is recommended for those working with animals. Individuals enrolled in the Animal Occupational Health Program should consult with their physician for obtaining tetanus boosters every ten years, or as recommended.

Personnel who work with human pathogens (rabies derived viral vectors as an example) should be offered the choice of receiving a vaccine, if available, and informed of the risks associated with the vaccine.

6.2 Preparing for potential exposures

Before beginning work with human pathogens, human blood or OPIM, all applicable safety information for a specific pathogen must be reviewed. Knowledge of exposure routes, symptoms and treatment methods will provide better preparation for the possibility of exposure to the human pathogen, human blood or OPIM being used. If exposure to a human pathogen, human blood or OPIM occurs or is suspected to have occurred while at work, the appropriate medical treatment should be sought immediately.

VII. Standard Microbiological Practices

7.1 Engineering Controls

Engineering controls are the preferred line of defense for protecting the worker and the environment from biohazards. Engineering controls include elimination of the hazard; facility design (e.g., directional airflow, self-closing doors, hands-free sinks); and safety equipment (e.g., biosafety cabinets) and enclosed containers, which are designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the primary device used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in BMBL's Appendix A, "Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets". More information on BSCs is provided in the following section of this manual.

7.2 Work Practices

Safe lab practice is critical to preventing exposure when working with rsNA and infectious materials. The best laboratory and safety equipment in the world cannot provide protection without good work practices and adequate training. The Principal Investigator should establish and model a laboratory culture emphasizing safe and responsible work practices.

7.3 Training and Education

Anyone planning to work with rsNA and infectious materials must be adequately trained before beginning the work. Supervisors are responsible for ensuring that all personnel receive proper training. **EHS provides general Lab Safety, Waste Management, Fire Safety, Biosafety Level 2 and Bloodborne Pathogens (BBP) trainings**. Annual refresher training is required for BBP and highly recommended for remaining topics to ensure continued safety. Lab Supervisors are required to provided Lab Specific training. Information communicated in the training should include:

- a discussion of this manual and how it applies to activities conducted in specific work areas
- an explanation of the health hazards, signs and symptoms of exposure to rsNA and infectious materials used in specific work areas
- a description of actions personnel can take to protect themselves from exposure, such as special work practices, use of a Biosafety Cabinet, location and use of safety equipment (eyewash, safety shower, first aid kit, etc.), vaccinations available, emergency procedures, etc.
- procedures to follow in case of an exposure or spill, including incident reporting, injury reporting and reporting requirements to outside entities (DEQ, OSHA, NIH for example).

Signed and dated documentation of training sessions and/or competency should be

maintained by both the supervisor and individual receiving training.

7.4 Signs and Labeling

Anyone entering areas where biohazardous rsNA and infectious materials are used must be aware of the potential hazards. Biohazard signs should be posted on doors to rooms where microorganisms, rsNA and/or biological toxins known to cause disease in humans are used, such as microorganisms classified as Biosafety Level 2 or greater. Red or orange BIOHAZARD labels should be placed on all containers and storage units (refrigerators, freezers, incubators, waste containers, etc.) that are used for microorganisms, rsNA or biological toxins causing disease in humans. Contaminated equipment must be labeled as well. Yellow animal biohazard signs should be posted where strict animal pathogens are used. Updates to emergency door signs may be requested from EHS.

7.5 Laboratory Practices and Techniques

Workplace-acquired infections do occur, and they are preventable. Information about the organism(s) should be gathered prior to commencing work with them. Good starting points for safety information about human pathogens are the Public Health Agency of Canada's Pathogen Safety Data Sheets and the Agent Summary Statements listed in the *BMBL*.

Infectious agents are transmitted through one or more of these routes of exposure:

- *Contact*: mucous membrane exposure (including the eyes, inside of the mouth and nose, and the genitals) or through broken skin (such as chapped or damaged skin through eczema, acne, etc.)
- *Injection*: via a puncture or cut with sharps (needlesticks, cuts with contaminated broken glass, etc.), also known as percutaneous exposure
- *Inhalation:* breathing in aerosols (microscopic solid or liquid particles small enough to remain dispersed and suspended in air for long periods; about 5 micrometers or less in diameter)
- *Ingestion*: by eating or drinking the contaminant

Using work practices that block routes of exposure can prevent workplace infection. Good microbiological techniques must always be used in the laboratory, as well as these standard practices:

- Eating, drinking, smoking, applying cosmetics or storing food in laboratories is strictly prohibited. Potentially contaminated hands should be kept away from the mouth, eyes, and non-intact skin.
- Hands should be washed frequently, even after wearing gloves, and scrubbed vigorously with soap and water for a full 30 seconds (as long as it takes to sing "Happy Birthday" or the "Oregon Fight Song"). The physical removal of organisms from the skin is just as important as using a disinfectant.
- Work surfaces and equipment must be decontaminated immediately after using rsNA or biohazardous materials, and routinely disinfecting items that may be handled by non-gloved hands.

- Wearing appropriate personal protective equipment (PPE) blocks potential routes of exposure. CDC/NIH BSL2 recommended PPE includes gloves, Lab coats or gowns, and eye and face protection (safety glasses, goggles, mask, face shield or other spatter guard).
- Keeping personal items such as cell phones and headphones out of lab areas.

More specific suggestions for common laboratory procedures used with biohazardous rsNA or infectious materials follow. Each prevents biohazardous rsNA or infectious materials from entering the body through common exposure routes.

Pipetting:

The greatest risks with pipetting are the creation of aerosols and splashing. Mouth pipetting is prohibited; mechanical pipetting aids should be used instead. All biohazardous rsNA or infectious materials should be pipetted in a biosafety cabinet. Cotton-plugged pipets should be used. Biohazardous rsNA and infectious materials must never be forcibly discharged from pipets. "To deliver" pipets should be used instead of pipets requiring blowout to reduce generating aerosol droplets. Disposable plastic pipettes are preferred, however if reusable pipettes are used, they should be placed horizontally in a pan filled with enough liquid disinfectant (not bleach) to completely cover them. The entire pan should be autoclaved before cleaning the pipets for reuse. Never autoclave a bleach solution as this results in the generation of toxic chlorine gas.

Sharps:

The greatest risks when using sharps are accidental injection. Needles and syringes should only be used when there is no alternative. Safety needles and syringes must be used when available and feasible. The sharp should be kept away from the fingers as much as possible. Sharps should never be bent, sheared, recapped, nor have needles removed from syringes after use. If a contaminated needle must be recapped or removed from the syringe, use a mechanical device, such as forceps, or recap using a one-handed scoop technique.

Sharps use also creates aerosols. Air bubbles should be minimized when filling a syringe. A pad moistened with disinfectant should be placed over the tip of the needle when expelling air. Work should be performed in a biosafety cabinet if it involves biohazardous or infectious materials. An appropriate sharps container must be kept close to the work area to avoid walking around with contaminated sharps. Care should be taken not to overfill sharps containers: they are considered full when they are 3/4 filled.

Vortexing, Blending, Grinding, Sonicating, Lyophilizing:

The greatest risk when using any of these devices is the creation of biohazardous or infectious aerosols. This equipment should be operated in a biosafety cabinet whenever possible. Safety blenders should be used. Safety blenders are designed to prevent leakage from the bottom of the blender jar and to withstand sterilization by autoclaving. They also provide a cooling jacket to avoid biological inactivation. Avoid glass blender jars; if a glass jar must be used, it must be covered with a polypropylene jar to contain the glass in case of breakage.

A towel moistened with disinfectant must be placed over the top of the blender while operating. This practice can be adapted to grinders and sonicators as well. Aerosols must be

allowed to settle for five minutes before opening the blender jar (or grinder or sonicator container). Lyophilizer vacuum pump exhaust should be filtered through HEPA filters or vented into a biosafety cabinet. Polypropylene tubes should be used in place of glass ampoules for storing biohazardous material in liquid nitrogen. Ampoules can explode, causing eye injuries and exposure to the biohazardous material.

7.6 Personal Protective Equipment

Appropriate PPE is chosen by considering the potential routes of exposure that need to be blocked to prevent exposure and infection. It is essential that PPE be removed before leaving the laboratory or animal room. PPE must never be taken home.

Lab Coats and Closed Shoes:

Lab coats, scrub suits, gowns, and closed shoes prevent hazardous materials from reaching skin, and more importantly, any cuts, dermatitis, etc. that may be present. They also protect street clothing from needing decontamination, as well as preventing contamination of laboratory cultures from the normal flora present on the skin. At minimum, a long-sleeved lab coat and closed-toe shoes must be worn in any microbiology laboratory. Long sleeves minimize contamination of skin and street clothes and reduce shedding of microorganisms from the skin. Closed shoes protect the feet from spills and injuries from dropped sharps.

Elastic-cuffed labcoats help prevent spills caused by catching the cuff on laboratory equipment. When working with rsNA or infectious materials inside a biosafety cabinet, elastic cuffs prevent contaminated air from being blown up the lab coat sleeve into the breathing zone. Lab coats must remain in the laboratory when personnel go home or when personnel move to non-laboratory work areas (such office, breaks rooms, communal spaces). This keeps any contamination in the laboratory instead of spreading it to other work areas or homes. Lab coats must not be taken home to be laundered. EHS provides lab coats to lab staff at no cost which also includes free laundering.

Gloves:

Gloves prevent exposure of the skin, and any cuts, dermatitis, etc. that may be present, to infectious materials. Nitrile gloves will prevent exposure to microorganisms. However, gloves must be compatible with the chemicals being handled, as well as offering protection from rsNA or infectious materials. EHS can provide assistance with choosing appropriate gloves. For the best protection, the cuffs of the gloves should overlap the cuffs of the laboratory coat. Disposable gloves must not be reused. They are designed for disposal after one use or if exposed to a chemical. Utility gloves, such as rubber dishwashing gloves may be disinfected for re-use if they do not show signs of wear or degradation. EHS can aid finding an alternative for those allergic to gloves (most common with latex) and/or the powder they contain.

Eye and Face Protection:

Eye and face protection prevent splashes into the eyes, nose and mouth (mucous membrane exposure), and onto the skin. Goggles or safety glasses should be worn to protect the eyes. Full-face shields should be worn to protect facial skin, such as when handling liquid nitrogen. Face masks protect against splashes, but do not prevent inhalation of aerosols. Face masks are

also useful in preventing lab exposures through splashes or accidental touching of the face.

Respirators:

Respirators prevent the inhalation of aerosolized microorganisms (inhalation exposure) when safety equipment designed to contain infectious aerosols, such as a biosafety cabinet, is not available. EHS can aid in determining the appropriate respirator needed. Respirator training and fit-testing is required for all respirators, regardless of required or voluntary use. Note: N95 are considered respirators and not masks. The EHS Respiratory Protection Program provides details. Respiratory protection as an alternative to engineering controls is not ideal and should only be implemented under the guidance of EHS.

7.7 Laboratory Safety Surveys

The UO Biosafety Checklist, used for surveys completed by EHS, includes criteria for work with infectious agents and for work with rsNA. Periodic self-auditing, using the criteria in Appendix G of the *NIH Guidelines* and Section IV of *BMBL,* of recommended Biosafety Level practices and containment will help ensure that good laboratory safety practices are being used.

7.8 Animal Handling

The spread of infectious agents between laboratory animal populations can be prevented and laboratory personnel can be protected from zoonotic agents by adhering to the following basic guidelines, required by Animal Care Services wherever animals are housed or used on campus. Safe practices that apply to all animal areas, regardless of biosafety level, include:

- Shoe covers must be worn, when specified, upon entering an animal room.
- All animal room doors must remain closed at all times, except for entering and exiting.
- Disposable gloves must be worn when handling animals, bedding or soiled cages.
- Disposable or washable outer garments (such as lab coats, gowns, coveralls) must be worn to protect personal clothing from contamination.
- Eating, drinking smoking, applying cosmetics and handling contact lenses in animal rooms or procedure rooms is prohibited.
- Hand contact with the nose, eyes, or mouth is strongly discouraged when working with animals.
- Hands must be washed with soap and water immediately after handling any animals or animal equipment, and before leaving the animal facility or laboratory.
- Extra caution must be taken with needles or other sharp equipment used with animals. Needles shall remain capped until ready to use, and then promptly and properly disposed.
- Handle only those species for which proper handling training has been provided can prevent injury.
- Any bites or other wounds must be washed immediately with soap and water and appropriate medical attention sought. Complete and submit Workplace Injury Report to EHS/SRS.

- Unauthorized persons are prohibited from entering animal rooms. Additional requirements may be specified for certain research studies.

Fieldwork involving wild animals requires adapting the basic animal infection control guidelines to the particular situation in the field. One of the major concerns with fieldwork is exposure to wild populations that might carry one or more zoonotic diseases. Personnel working in areas where they are likely to be exposed to wild rodents or their nesting areas must consult with EHS on occupational health and safety considerations.

7.9 Cell and Tissue Culture

Cell cultures may contain viruses, and no cell culture line, even if purchased, can be definitively determined to be free of any and all potential pathogens. It is prudent to consider all cell lines to be potentially infectious. Most cell cultures can be safely manipulated using BSL2 practices and containment. If cells are known or suspected to contain a specific pathogen or oncogenic virus, appropriate biosafety practices for handling that virus must be used when working with the cell culture. **All other primary and permanent cell lines must be handled using BSL2 practices and containment.**

VIII. Biosafety Equipment

8.1 Biological Safety Cabinets (BSCs)

The BSC is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, III) and the horizontal laminar flow cabinet are described below. The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns or greater with an efficiency of 99.97%. However, it does not remove vapors or gases; this is why BSCs should never be used for work with volatile chemicals or flammable gases.

The BSC requires regular maintenance and certification by an NSF-accredited technician to ensure that it protects you, your experiments, and the environment. Each cabinet must be certified after installation, each time it is moved or repaired, and at least annually. If a BSC needs to be relocated, it must first be decontaminated by a certified contractor. Once the BSC has been moved, it must be recertified before use and annually thereafter. Additionally, repairs must only be made by certified contracted technicians. Costs for annual certification, decontamination, repairs or replacement of the HEPA filter is the responsibility of the user/owner. EHS can provide contact information for certified vendors. EHS administers this program for the University. Contact the BSO to confirm that your cabinet is included in this program.

8.2 Types of Biosafety Cabinets

BSC Type	Features

Class I Biosafety Cabinets	Protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.
Class II Biosafety Cabinets (Types A1, A2, B1, B2)	Provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).
Class III Biosafety Cabinets (sometimes called Class III glove boxes)	These were designed for work with infectious agents that require BSL4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through two HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.
Laminar flow "clean air benches"	These are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user's face, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

8.3 Operation of Class II BSCs

Step	Procedure
1.	Turn on cabinet fan 15 minutes before beginning work. Put on appropriate PPE: gloves at a minimum, and preferably a lab coat and safety glasses as well.
2.	Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
3.	Place supplies in the cabinet. Locate container inside the cabinet for disposal of

pipettes and other waste. (Movement of hands in and out of the cabinet to discard items into an outside container disrupts the air barrier that maintains sterility inside the cabinet.) Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids. Do not work in a BSC while a warning light or alarm is signaling.

4. Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a plastic bin) to prevent spilling.

5. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.

6. While working in a BSC, use slow, deliberate motions and minimize entering and exiting the cabinet. All waste and/or disinfecting containers must be kept inside the cabinet while they are being used.

7. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.

Additionally, the following guidelines should be followed with regard to proper BSC use:

- Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. In fact, **UV disinfection is discouraged because it is often impeded by shadows, dust, and inadequate intensity**. UV radiation should never take the place of a disinfectant for disinfection of the cabinet interior. The UV lamp should never be on while an operator is working in the cabinet.
- Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.
- Open flames are not necessary in a BSC. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence, which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames and gas burners is strongly discouraged in biosafety cabinets.** When deemed absolutely necessary, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

8.4 Vacuum Lines

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, a hydrophobic vacuum line filter should be used.

Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- If a glass flask is used at floor level, place it in a sturdy plastic container to prevent breakage by accidental kicking.
- In BSL2 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

Vacuum Line Filter

Adding a hydrophobic filter, between C & D in diagram below, will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Vacushield are available from several scientific supply companies.

A) collection flask containing disinfectant.

B) fluid overflow flask.

C) in-line HEPA Filter

D) vacuum system

8.5 Centrifuges

The greatest risk with centrifuging is the creation of aerosols. Centrifuge safety buckets and sealed rotors protect against release of aerosols. To avoid spills from broken tubes, the tubes, O-rings and buckets should be inspected for damage before each use. Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full. The outside of the tubes should be wiped with disinfectant after they are filled and sealed. Rotors and centrifuge tubes should be opened inside a biosafety cabinet. If a BSC is not available, a minimum of 10 minutes' settling time should be allowed before opening.

8.6 Autoclaves

Autoclaves are classified as pressure vessels and must be inspected at least annually according to Oregon Administrative Code, Section 875, Chapter 209. Repairs to most autoclaves on campus are done by Campus Planning & Facilities Management. Because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Injuries can be prevented when using the autoclave by observing the following rules:

- Heat-resistant gloves, eye protection and a lab coat must be worn, especially when unloading the autoclave.
- Steam burns and shattered glassware can be prevented by ensuring that the pressure in the autoclave chamber is near zero before opening the door at the end of a cycle. The autoclave door should be cracked open slowly to allow the steam to escape gradually.
- Items must be allowed to cool for 10 minutes before removing them from the autoclave.
- Sealed containers must never be put in an autoclave. They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.
- Solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), and radioactive materials must never be placed in an autoclave. EHS can provide assistance with any questions about proper disposal of these materials.
- Autoclave components must be inspected regularly. In particular, cleaning the drain screen frequently will help to prevent operation problems and down time. If a problem is discovered, repair must be initiated. An autoclave should never be operated until it has been properly repaired.

Due to infectious waste management requirements under the Oregon Health Authority, autoclaves at UO cannot be used to decontaminate infectious or biohazardous materials prior to disposal. These materials MUST be incinerated. Contact EHS to obtain incineration boxes and biohazard bags. UO autoclaves can be used to destroy non-infectious recombinant organisms (such as K12 E. coli, non-infectious yeast, fruit flies and nematodes).

IX. Disinfection

Sterilization, disinfection, and antisepsis are all forms of decontamination. *Sterilization* implies the killing of all living organisms. *Disinfection* refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. *Antisepsis* is the application of a liquid antimicrobial chemical to living tissue.

9.1 Chemical Disinfectants

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has

occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

- *Sterilizer or Sterilant:* will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
- *Disinfectant:* will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
- *Hospital Disinfectant*: agent shown to be effective against *S. aureus*, *S. choleresis* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
- *Antiseptic*: agent formulated to be used on skin or tissue - not a disinfectant.

9.2 Disinfectants Commonly Used in the Laboratory

Hypochlorites (bleach)

- Working dilution is 1:10 to 1:100 household bleach in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- 10 minutes minimum contact time.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made monthly.

Iodophors (iodine, Wescodyne)

- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- 10 minutes minimum contact time.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- Stable in storage if kept cool and tightly covered.
- Built-in color indicator; if solution is brown or yellow it is still active.
- Relatively harmless to humans.

Alcohols (ethanol, isopropanol)

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting. 30 second wet contact time.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

9.3 Dilution of Disinfectants

Chlorine compounds (Household Bleach)

Dilution in Water	% Available Chlorine	Available Chlorine (mg/l or ppm)

Undiluted	5.25	50,000
1:10	0.5	5,000
1:100	0.05	500

Bleach solutions decompose at room temperature and should be made fresh ***monthly***. Date all containers of diluted bleach solution including spray bottles and aspirator trap flasks. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 10%. Bleach use on stainless steel surfaces should be followed by a rinse and wipe using 70% ethanol to remove corrosive residues. Shelf-stable pre-diluted bleach solutions may be preferable for ease of use and storage

Iodophors

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons of water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons of water.

Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant. Surprisingly, stronger solutions of ethanol (90%) are *less* effective.

X. Biological Waste Disposal Procedures

Lab staff must request pickup of hazardous wastes through EHS. The University of Oregon is unique in that the wastes generated are of an extremely varied nature. Researchers, instructors, and support services generate much of the University's hazardous waste. Everyone at the University who generates hazardous waste has the responsibility to ensure the success of the Hazardous Waste Management Program. The priorities of the program include:

- Reducing the quantity of hazardous waste generated
- Managing hazardous waste in a manner which protects the health and safety of students, staff, and faculty at the University, as well as the surrounding community
- Managing hazardous waste using the most environmentally sound and responsible methods practical
- Lowering the potential for a release of hazardous waste into the environment
- Complying with governmental regulations regarding hazardous waste management

10.1 Biological Waste

Pathological waste must be separated from non-pathological biohazardous waste at point of generation. Decontamination prior to disposal is required for materials that are potentially infectious or recombinant. Pathological waste is defined as human or animal bodies, body

parts, organs or tissues must be disposed by incineration. **If there is a question as to if a material is biohazardous, recombinant, or pathological and how to properly dispose, please contact EHS for clarification.** Solid biological waste materials must be directly disposed into red-bagged lined Biohazard waste boxes obtained from EHS. When biohazard containers are almost full or have reached 45 pound weight limit, submit an online waste pickup request. Alternatively, the groups may collect these materials in sturdy trash bins labeled with the biohazard symbol, and lined with bags printed with a biohazard symbol. These bags are picked up by EHS. Waste defined as

10.2 Multi-hazard or Mixed Waste

Mixed waste is defined as waste containing more than one hazard. Avoid generating mixed waste if possible, and keep volume to minimum. Do not autoclave mixed waste.

When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, then dispose as radioactive waste. Seek advice from the RSO before beginning inactivation procedures.

When discarding waste containing an infectious agent and a hazardous chemical, dispose as chemical waste. Contact EHS for advice.

10.3 Animal Tissues, Carcasses and Bedding

Pathological waste, animal and human tissues, organs, body parts, is to be collected by lab staff in separate plastic bags with Biohazard symbol and labeled “Pathological Waste” and stored in freezer until collected by EHS. Small amounts of pathological waste can be combined in a larger pathological waste bag. Once significant amount of pathological waste has been collected (1-2 gallon bag) submit online waste pick up request and select “Pathological” waste type from pull down menu.
Bedding from infected animals should be disposed of as “Biohazard” as described above.

10.4 Autoclaving rsNA Waste

Autoclaves are to be used at **UO *only* for destroying non-pathogenic recombinant materials (recombinant E. coli, yeast, fruit flies and nematodes), NOT biohazardous materials**. If you are unsure how your waste should be decontaminated, please contact the UO Biosafety Officer.

Autoclaves use pressurized steam to destroy microorganisms and are commonly used for the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

Container Selection

Polypropylene bags, commonly called autoclave bags, are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, **place bags in**

a rigid container such as a polypropylene or stainless-steel pan during autoclaving. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed. Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.

Always place bags into bins before loading into the autoclave. Polypropylene containers and pans are a plastic capable of withstanding autoclaving, but are resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless-steel pan. To decrease the time required to sterilize material in these containers,

- remove the lid (if applicable)
- turn the container on its side when possible
- select a container with the lowest sides and widest diameter possible for the autoclave.

Stainless steel containers and pans are an alternative to polypropylene bins. Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though it is more expensive than polypropylene.

Preparation and Loading of Materials

- Fill liquid containers only half full.
- Loosen caps or use vented closures.
- Always put bags of biological waste into autoclavable pans to catch spills.
- Position autoclave bags on their sides, with the bag neck taped loosely.
- Apply a piece of autoclave indicator tap to the bag.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless-steel pans.

Cycle Selection

- Use liquid cycle when autoclaving liquids, to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.
- Use fast exhaust and dry cycle for wrapped items.

Time Selection

- Bags of non-pathogenic biological solid waste should be autoclaved for 60 minutes at 121°C and 15 psi to assure decontamination.
- Consider the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.

Removing the Load

- Check that the chamber pressure is zero.

- Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware of rush of steam.
- After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.
- All autoclaved bags of waste must be put into plain opaque household trash bags before being put into the dumpster.

Monitoring

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121°C, and 2) heat-resistant spores (*Bacillus stearothermophilus*) that are killed by exposure to 121°C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the densest point.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121°C. Ampules of heat-resistant spores should be used in subsequent test runs to determine the length of time necessary to achieve sterilization.

Autoclaving Reusable Labware

Items such as culture flasks and centrifuge bottles are decontaminated by lab personnel before washing by either 1) autoclaving items in an autoclavable container, or 2) chemically disinfecting items by soaking in diluted disinfectant for one hour before washing.

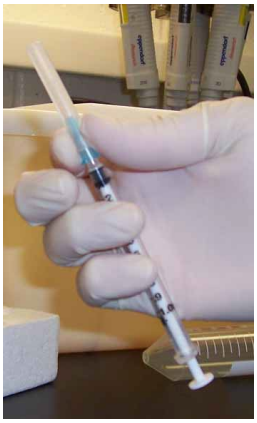
10.5 Sharps

Sharps include all materials capable of puncturing the skin. Examples include needles, razor blades, scalpels, glass (Pasteur pipettes, slides and coverslips, etc). Sharps are a leading cause of lab injuries and should be eliminated from lab procedures whenever possible. When this is not feasible, consider replacements: using plastic materials instead of glass and using safer sharps devices. To prevent needlestick injuries:

- Eliminate the use of sharps in your work or replace glass with plastic.
- Do not bend, break, or otherwise manipulate needles by hand.
- Do not recap needles by hand.
- When needles must be removed from syringes, do not remove them by hand; use hemostats.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Use extra care when two persons are working together. Locate sharps container between the workers when possible.

Recapping

Occasionally needles must be filled, recapped, and set aside for use later, such as when preparing for an animal injection. In these cases, recapping may be performed by the one-handed scoop technique, or by placing the needle in a sterile conical tube:



All Other Sharps

The State of Oregon defines "sharps" to include the following:

- Needles
- IV tubing with needles attached
- Scalpel blades (including razor blades)
- Lancets
- Glass tubes that could be broken during handling (e.g., capillary tubes, thin-walled test tubes, Pasteur pipettes)
- Syringes that have been removed from their original sterile containers

Additionally, a "syringe" is an instrument that consists of a hollow barrel fitted with a plunger and a hollow needle.

UO requires that all sharps be collected in one of the following containers:

1. *In areas used for patient care, Animal and Biosafety Level 2 laboratories, or otherwise used with human or non-human primate source materials*, a standard sharps container containing a biohazard symbol, as depicted below.

2. In all other areas, such as Animal and Biosafety Level 1 laboratories, chemistry laboratories, arts/theater, and facility maintenance use*, the sharps containers depicted above are*

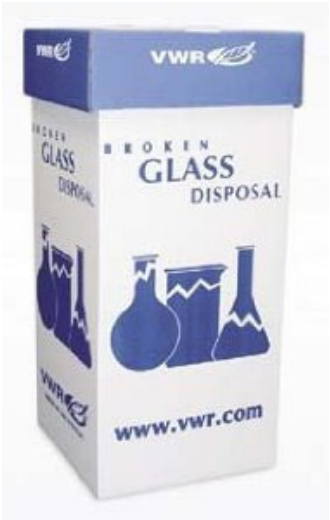
optional. Alternatively, groups may collect sharps in an EHS-approved red, leakproof, rigid, puncture-resistant container that can be securely closed once filled. The container must clearly be marked "SHARPS" and conflicting information removed or marked through. Examples include plastic laundry detergent bottles, coffee cans, or containers intended for non-hazardous sharps (below)

When containers are ¾ filled, close the lid securely and request pickup from EHS. **ALL** sharps must be picked up by EHS for disposal, with the only exceptions being groups contracting directly with a waste disposal vendor. Do not place sharps in the dumpster.

Syringes without attached needles may be disposed of in the biohazard (red bag) waste stream. However, removing needles from syringes for the express purpose of preferred waste disposal is prohibited.

10.6 Glass

Laboratory glassware (e.g., beakers, Erlenmeyer flasks) and broken glass that is not contaminated with hazardous materials must be disposed of in standard "Broken Glass" boxes. These boxes may be obtained from Science Stores or scientific supply companies. The boxes should be securely taped at the bottom to prevent heavy contents from breaking through, and the plastic bag liner must be in place. Use of homemade cardboard boxes or other containers is only permitted with express approval from EHS. Must clearly be labelled "Broken Glass Only".



Glass contaminated with infectious or recombinant materials may be decontaminated with appropriate disinfectant and then disposed in the "broken glass" box IF the glass can be safely handled. Alternatively, it may be disposed of in the sharps container.

If glassware is contaminated with chemical or radiological material, please consult with EHS for disposal options.

When the box is filled, please tape it shut securely and dispose into the nearest dumpster. Unbroken, empty reagent containers are not considered laboratory glassware, and may be disposed directly to garbage dumpsters.

XI. Lentiviral Vectors

The use of lentiviral vectors has been increasing because the vector system has attractive features; however, they are not risk-free. It is important to remember that **replication-incompetent does not mean non-infectious**.

The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration.

The nature of the transgene must also be considered in assessing risk. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a constitutive strong promoter may require heightened safety precautions).

The potential for generation of replication-competent lentivirus from HIV-1 based lentiviral vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentiviral vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. (It should be noted, however, that pseudo typing with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentiviral vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3' LTR that renders the vector "self-inactivating." In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for becoming replication-competent.

The most probable route of exposure for this work would be percutaneous via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation of aerosols depending on the use of equipment such as centrifuges or vortex mixers. Care must be taken when pipetting in order to avoid splashing or generation of aerosols. There may be increased risk potential for HIV positive individuals whose native virus may recombine with the recombinant virus. Such individuals are encouraged to discuss this with their physician. Immunocompromised individuals should not work with lentivirus.

You will need to provide a comprehensive risk assessment considering the nature of the vector system, transgene insert, vector propagation, and if applicable, animal hosts and

manipulations, as part of your IBC registration. For many experiments, it is appropriate to use either BSL-2 or enhanced BSL-2 containment and practices with elimination/restrictions on sharps use.

XII. Transporting Infectious & rsNA Materials

12.1 General Information

You must attend a training class before you package infectious substances (human or animal pathogens) for transport by commercial carrier. The U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) regulate shipment of human and animal pathogens. The regulations are complex and exacting. They require that researchers who prepare infectious materials for shipment receive training every two years. In addition, packages must be marked and labeled exactly as the regulations specify, and packaging materials must have been tested and certified to withstand certain durability and pressure tests. Cardboard boxes in which supplies have been received cannot be used to ship infectious materials. Recent events have led to greater scrutiny for compliance with these regulations.

12.2 Permits

Permits are required from the Centers for Disease Control and Prevention (CDC) to **import or transport** 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least ten working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 718-2093, or through the CDC web site at https://www.cdc.gov/import-permit-program/php/.

Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for **importation or domestic transport of agents** infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained through the APHIS web site at https://www.aphis.usda.gov/organism-soil-imports.

Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests.** Application should be made at least 120 days in advance of the anticipated release or

shipment date. Information and application forms may be obtained through the APHIS web site.

A validated license is required by the Department of Commerce Bureau of Industry and Security for **export** of certain microorganisms and toxins (listed in 15 CFR Part 774) to all destinations except Canada. Information may be obtained by calling (202) 482-0896.

There is also a lot of helpful information on the UO Export Controls web page https://research.uoregon.edu/manage/export-controls.

12.3 Packaging

Various carriers (FedEx, UPS, US Postal Service or others) have different requirements for packaging and labeling infectious substances, and some carriers refuse to accept certain materials. In addition, various agencies such as the International Air Transport Association (IATA), and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances. Therefore, it is important to check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and/or labeling.

- Infectious Substance: a viable microorganism, or its toxin, which causes or may cause disease in humans.
- Diagnostic Specimen: any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- Biological Product: a product for human or veterinary use, such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen;
- A secondary container that contains the primary container and packaging capable of absorbing the specimen; and
- An outer rigid shipping container that contains the secondary container and other material.

12.4 Genetically Modified Microorganisms

The *International Air Transport Association's Dangerous Goods Regulations* outlines requirements for shipment of GMOs. Genetically-modified Category A agents must be shipped as Category A. Genetically-modified Category B agents must be shipped as Category B. Non-pathogenic GMOs are exempt from the shipping regulations.

12.5 Human Clinical Materials

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard

symbol or color-coded. Clinical samples with a reasonable likelihood of carrying pathogens fall under the IATA regulations as well. Various carriers may have additional requirements.

12.6 On-Campus Transport

Any infectious or rsNA materials transported between laboratories or buildings on campus should be contained, as they would be in the laboratory, to prevent release of the materials into the environment. Transport containers should be labeled with the biohazard symbol and the identity of the material inside. For example, to transport a rack of test tubes containing Risk Group 2 organisms from a laboratory in Streisinger to Klamath, the tubes should be capped and placed inside a sealed, puncture-resistant, unbreakable secondary container with a biohazard label. The secondary container must remain intact in the event it is dropped.

12.7 Select Agent Human Pathogens and Biological Toxins

The Department of Health and Human Services rule "Additional Requirements for Facilities Transferring or Receiving Select Agents," which expanded the regulations that were already in existence, went into effect in 1997. Facilities sending out or receiving certain designated Select Agents, such as certain specified viruses, bacteria, rickettsia, fungi and biological toxins, are now required to apply for and receive a Site Registration Number from the CDC before any shipments occur. Substantial criminal penalties apply to both individuals and organizations that do not comply with the regulation requirements. The University of Oregon currently does not hold, use or transfer threshold quantities of Select Agent Toxins. Any Principal Investigator anticipating use of select agents or greater-than-permissible amounts of select toxins must contact EHS *immediately* for initiation of a select agent program at UO. This process is extensive and complicated, and requires at least a year of lead time prior to receipt of the material.

12.8 Off-Campus Transport by Non-Commercial Methods

Please refer to UO's guidance on Hazardous/Regulated Materials Transportation. Materials must be classified, packaged, and labeled the same as if they were being transported via commercial courier. UO personnel may transport infectious or rsNA materials by non-commercial routes only in university-owned vehicles. Personal vehicles may not be used due to insurance coverage limitations. Transport by non-commercial routes may only be done within the state of Oregon.

XIII. Laboratory Security Considerations

Although most microbiology laboratories contain a variety of dangerous biological, chemical and radioactive materials, these materials have rarely been used to intentionally injure anyone. However, there is growing concern about the possible use of biological or rsNA, chemical and radioactive materials by terrorists. In response to these concerns, the CDC has included guidelines to address laboratory security issues in the current edition of *Biosafety in Microbiological and Biomedical Laboratories*. Security is most critical for laboratories using

biological agents, rsNA or toxins capable of causing serious or fatal illness to humans or animals, or causing serious damage to indigenous plants. All laboratories, however, should consider the following basic points of security and how they might apply to their individual situations. All laboratory personnel are responsible for:

- controlling access to areas where infectious agents, rsNA or toxins are used and stored, and for locking refrigerators/freezers used to store agents
- knowing who is in the laboratory
- knowing what materials are brought into the laboratory
- knowing what materials are removed from the laboratory
- reporting any undocumented visitors; missing biological, rsNA, chemical or radioactive materials; unusual or threatening phone calls; and suspicious persons or packages to the laboratory supervisor, EHS, and campus police

Appendix A - Applicable Regulations and Guidelines

The following federal and international regulations and guidelines apply to work performed with biological materials:

- NIH Guidelines for Research Involving Recombinant DNA Molecules (04/2024):

http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

- NIH Office of Science Policy: https://osp.od.nih.gov/
- Biosafety in Microbiological and Biomedical Laboratories (6th edition): https://www.cdc.gov/labs/bmbl/
- University of Oregon Biological Research Registration Forms:

https://safety.uoregon.edu/institutional-biosafety-committee

- Oregon OSHA Bloodborne Pathogen Standard:

http://osha.oregon.gov/Pages/topics/bloodborne-pathogens.aspx

- USDA/APHIS Permitting Requirements:

https://www.aphis.usda.gov/aphis/resources/permits

- CDC Import Permit Program: https://www.cdc.gov/import-permit-program/php/
- Federal Select Agent Program: http://www.selectagents.gov/
- Export Administration Regulations Commerce Control list (15 CFR Part 774):

http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=02b534f76f96c9c5f13a1d1be8edf8d4&ty=HTML&h=L&n=15y2.1.3.4.45&r=PART

- NSF Standard 49 for the Evaluation of Class II Laminar Flow Biological Safety Cabinets:

https://www.nsf.org/lab-testing/biosafety-cabinetry/biosafety-cabinet-certification

University of Oregon Safety- Physical Space and Environment Policy

- https://policies.uoregon.edu/vol-4-finance-administration-infrastructure/ch-5-public-safety/safety-physical-space-and-environment

Principles of Good Microbiological Practice

1. Never mouth pipette. Avoid hand to mouth or hand to eye contact in the laboratory. Never eat, drink, apply cosmetics or lip balm, handle contact lenses or take medication in the laboratory.
2. Use aseptic techniques. Hand washing is essential after removing gloves and other personnel protective equipment, after handling potentially infectious agents or materials and prior to exiting the laboratory.
3. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) recommends that laboratory workers protect their street clothing from contamination by wearing appropriate garments (eg, gloves and shoe covers or lab shoes) when working in Biosafety Level-2 (BSL-2) laboratories. In BSL-3 laboratories the use of street clothing and street shoes is discouraged; a change of clothes and shoe covers or shoes dedicated for use in the lab is preferred. BSL-4 requires changing from street clothes/shoes to approved laboratory garments and footwear.
4. When utilizing sharps in the laboratory, workers must follow OSHA!" Bloodborne Pathogens standard requirements. Needles and syringes or other sharp instruments should be restricted in laboratories where infectious agents are handled. If you must utilize sharps, consider using safety sharp devices or plastic rather than glassware. Never recap a used needle. Dispose of syringe-needle assemblies in properly labeled, puncture resistant, autoclavable sharps containers.
5. Handle infectious materials as determined by a risk assessment. Airborne transmissible infectious agents should be handled in a certified Biosafety Cabinet (BSC) appropriate to the biosafety level (BSL) and risks for that specific agent.
6. Ensure engineering controls (e.g., BSC's, eyewash units, sinks, and safety showers) are functional and properly maintained and inspected.
7. Never leave materials or contaminated labware open to the environment outside the BSC. Store all biohazardous materials securely in clearly labeled, sealed containers. Storage units, incubators, freezers or refrigerators should be labeled with the Universal Biohazard sign when they house infectious material.
8. Doors of all laboratories handling infectious agents and materials must be posted with the Universal Biohazard symbol, a list of the infectious agent(s) in use, entry requirements (e.g., PPE) and emergency contact information.
9. Avoid the use of aerosol-generating procedures when working with infectious materials. Needle clipping, pipetting mixing, sonication, and centrifugation can produce substantial aerosols. If you must perform an aerosol generating procedure, utilize proper containment devices and good work practice controls to mitigate potential exposures; Tightly cap tubes prior to centrifuging or vortexing; Allow aerosols to settle prior to opening tubes, equipment; Open tubes or equipment inside a containment device whenever feasible; Shield instruments or activities that can emit splash or splatter.
10. Use disinfectant traps and in-line filters on vacuum lines to protect vacuum lines from potential contamination.

11. Follow the laboratory biosafety plan for the infectious materials you are working with and use the most suitable decontamination methods for decontaminating the infectious agents you use. Know the laboratory plan for managing an accidental spill of pathogenic materials. Always keep an appropriate spill kit available in the lab.
12. Clean laboratory work surfaces with an approved disinfectant after working with infectious materials. The containment laboratory must not be cluttered in order to permit proper floor and work area disinfection.
13. Never allow contaminated, infectious waste materials to leave the laboratory or to be put in the sanitary sewer without being decontaminated or sterilized. When autoclaving use adequate temperature (121 C), pressure (15 psi), and time, based on the size of the load. Also use a sterile indicator strip to verify sterilization. Arrange all materials being sterilized, so as not to restrict steam penetration.
14. When shipping or moving infectious materials to another laboratory, always use U.S. Postal or Department of

    Transportation (DOT) approved, leak-proof sealed and properly packed containers (primary and secondary containers). Avoid contaminating the outside of the container and be sure the lid is on tight. Decontaminate the outside of the container before transporting. Ship infectious materials in accordance with Federal and local requirements.
15. Report all accidents, occurrences and unexplained illnesses to your work supervisor and the Occupational Health Physician. Understand the pathogenesis of the infectious agents you work with.
16. Think safety at all times during laboratory operations. Remember, if you do not understand the proper handling and safety procedures or how to use safety equipment properly, do not work with the infectious agents or materials until you get instruction. Seek the advice of the appropriate individuals. Consult the CDC/NIH BMBL for additional information. Remember, following these principles of good microbiological practices will help protect you, your fellow worker and the public from the infectious agents you use.

This Fact Sheet was developed as a product of the OSHA and American Biological Safety Association Alliance for informational purposes only. It does not necessarily reflect the official views of OSHA or the U.S. Department of Labor.

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Stability of Free Available Chlorine Levels in Dilute Sodium Hypochlorite Solutions over a 6-Week Period

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Animal care and use programs commonly use chlorine and chlorine-based disinfectants to help prevent facility acquired infections in animals. The Department of Comparative Medicine (DCM) at Oregon Health and Science University (OHSU) follows the Centers for Disease Control and Prevention (CDC) disinfection guidelines for preparing and storing these disinfectants. DCM prepares bottles of dilute solutions of sodium hypochlorite (that is, commercial bleach) daily. In this study, we tested whether dilute bleach solutions, as prepared following the DCM protocol, remained stable under real-world practice conditions for up to 6 wk. We tested 4 groups of spray bottles filled with 0.5% bleach solutions in these experiments. Specifically, we sprayed 2 groups of bottles daily to mimic use while 2 other groups of bottles were not sprayed. We then measured free available chlorine (FAC) using 2 methods, spectrophotometry and colorimetric strips. All 4 test groups showed stable maintenance of FAC concentration for the length of the experiment. Mean FAC loss from baseline levels was not significantly different in the group of bottles not sprayed daily (6% for group 2 at week 5 compared with 7% for Group 4 at week 6). All bottles in Groups 1 and 3 measured by colorimetric strips showed concentrations at or near 5000 mg/L at all weekly time points throughout the experiment. This study shows that 0.5% sodium hypochlorite solutions stored and used in a standard rodent housing room and sprayed daily will maintain acceptable FAC concentrations for at least 5 to 6 wk, perhaps longer. In addition, we report that colorimetric strips may be a useful and accessible quality control tool for testing freshly prepared solutions at regular intervals. We conclude that sodium hypochlorite solutions can be prepared on a weekly, biweekly, or monthly basis with no loss in disinfection effectiveness.

Abbreviations: DCM, Department of Comparative Medicine; OHSU, Oregon Health and Science University; CDC, Centers for Disease Control and Prevention; FAC, free available chlorine

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Introduction

The prevention of animal infection through the accidental introduction or propagation of pathogens is a high priority in a research setting. Infections in research animals can spread quickly and confound studies, and may require diagnostic tests, isolation, or treatment of infected animals. Some pathogens may require colony depopulation to ensure the long-term health of animals in a facility. Laboratory animal medicine also presents unique challenges to infection control as animals with varying degrees of immune status are routinely used in research experiments. Immunocompromised animals are more susceptible to infection and are more likely to require euthanasia due to poor health.

Infectious microorganisms can be introduced easily through mishandling or improper disinfection of animal housing or care items, but can be successfully avoided using disinfection protocols from the CDC with Environmental Protection Agency (EPA)-registered disinfectants, such as alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, hydrogen peroxide, iodophors, phenolics and quaternary ammonium compounds.[18] Disinfectant effectiveness (that is, spectrum of activity, speed of action) must be balanced against health risks and financial costs. Some disinfectants, while effective, may cause animal toxicity, including lung and brain injury from fume inhalation or occupational health hazards such as fume inhalation, dermal or ocular irritation or burns, the cost of disinfectant and of the labor required to prepare and use it must also be considered.[18] Moreover, the CDC offers guidelines for disinfectant use in human healthcare facilities, but these may not be appropriate for animal research settings. CDC guidelines on disinfectant concentrations and use may be excessively stringent for carefully controlled laboratory environments, causing unnecessary expense to programs and potential harm to animals and staff. Alternatively, disinfectant storage conditions in research environments may be harsher than healthcare environments, speeding degradation and decreasing effectiveness more quickly and suggesting that more stringent protocols are needed.

Many care and use programs for research animals follow CDC guidelines and use chlorine-based disinfectants, particularly sodium hypochlorite (bleach), for routine workstation and animal equipment disinfection. In this study, we evaluated the standard operating procedure (SOP) for sodium hypochlorite disinfection in the DCM at OHSU. Per departmental SOP, we prepared a 0.5% (5000 mg/L) working solution daily for the disinfection of rodent workstations, transfer forceps, equipment and surfaces that may contact rodents, their caging or their waste. This SOP is based on current CDC guidelines and represents current use of the guidelines in a representative animal care and use program.

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The DCM uses bleach because of its reasonably broad spectrum of antimicrobial activity, low toxicity and levels of toxic residues, lack of sensitivity to water hardness, quick action, and low cost. However, some small but notable differences between CDC recommendations and current practice argue for a more stringent protocol. For example, the CDC Disinfection Guidelines state that, "hypochlorite solutions in tap water at a pH >8 stored at room temperature (23°C) in closed, opaque, plastic containers can lose up to 40-50% of their free available chlorine (FAC) over one month."[18] OHSU DCM staff store dilute sodium hypochlorite solutions in opaque spray bottles in animal care rooms or at animal care stations in spaces with fluctuating temperature and light exposure. In addition, tap water pH is not routinely measured when DCM staff prepare dilute solutions. However, current OHSU DCM protocols could be overly stringent, particularly with regard to the requirement to prepare solutions fresh daily, which increases risk to DCM staff and costs for reagent and labor. The potential harms of sodium hypochlorite use are highest for the technical staff who prepare working dilutions from purchased commercial bleach (5000 mg/L); these risks include potential for ocular irritation and chemical burns, metal corrosion at high concentrations (>500 ug/L), potential release of chlorine gas when mixed with ammonia or acid, and low relative stability over very long time periods.[18] Therefore, we sought to determine whether the current CDC guidelines are appropriate for use in a laboratory animal setting.

In this study, we tested whether dilute bleach solutions, as prepared using the DCM protocol, remained stable under real-world practice conditions for up to 6 wk. The overall goal of this project was to provide practical guidance in use and storage of bleach solutions in research animal settings and to provide programs with technical information on testing options available for determining the stability of sodium hypochlorite used under specific storage and use conditions.

Prior studies have shown that the decomposition rate of sodium hypochlorite is mainly dependent on pH, concentration, temperature, and ambient light exposure.[5,8-11,15-17] The ideal storage conditions to maximize sodium hypochlorite solution stability would include maintaining a solution at a pH of 9 to 11, at temperatures below 30 °C, and in an opaque bottle with little to no ambient light exposure.[4-6] One predictive model of FAC loss showed that a 1.25% commercially available sodium hypochlorite solution, stabilized to a pH of 11.9, degrades 10% after 660 d at 25 °C.[13] However, this model does not consider real-world variables. We added to the existing literature by testing the role of air introduction into bleach spray bottles due to daily use (via using spray bottles to mimic daily practice) and by testing for the loss of FAC over a 6-wk period when stored and used in an active laboratory animal care facility. We used 2 methods to determine FAC concentrations—spectrophotometry, a highly quantitative approach that may not be available to some programs, and colorimetric strips, which are widely available and easy to use but semiquantitative. We hypothesized that we would find be no significant difference in FAC concentrations over a 6-wk period, and that a difference in FAC concentrations would not develop between bottles sprayed daily and those that are not. If true, these results would indicate that dilute bleach solution remains stable for up to 6 wk under active use conditions, suggesting that less frequent solution preparation would be acceptable, representing a saving of both cost and effort.

Materials and Methods

Experimental design. We tested 4 groups of 3 spray bottles each (total of 12 bottles) of sodium hypochlorite solution (Figure 1). Two of the 4 groups were sprayed daily to mimic daily use; the remaining 2 groups were not sprayed (Figure 1). We then used 2 testing methods to evaluate FAC available chlorine concentrations at weekly time points: spectrophotometry and colorimetric chlorine strips.

The plastic spray bottles we used in these experiments were 32-ounce, white, opaque, high-density polyethylene bottles (Wesco Supply, Long Beach, CA) with 9 to 3/4" Adjust-O-Spray triggers (Wesco Supply, Long Beach, CA). To closely mimic practice conditions in rodent housing rooms, we stored bottles in a vacant rodent housing room and maintained these rooms on a 12:12-h light: dark cycle, at 19.4 to 22.8 °C and 30% to 70% relative humidity (as recorded by a thermo-humidity meter) for the duration of the study. Because light exposure has been previously shown to contribute to sodium hypochlorite degradation, we ensured maximum light exposure to simulate degradation under the most extreme practice conditions.[2,5,8,9,11,16,17] To accomplish this, we positioned bottles in a grid in the middle of a stainless-steel table, approximately 1 m high, directly below a lighting banister fitted with a compact fluorescent bulb (Figure 2). We adjusted the exact positioning under the light banister to expose all bottles to the same average light intensity (lux), as confirmed by measuring the center of the bottle grouping with a LX1330B lux meter (Sinometer, ShenZen, China).

Sodium hypochlorite solutions. In initial experiments, each bottle was filled with a 0.5% (5000 mg/L) sodium hypochlorite solution using the current DCM protocol. The solutions were made by diluting 83 mL of 6% Pure Bright Germicidal Ultra Bleach (KIK International LLC, Concord, Ontario, Canada) with 917 mL of tap water (1:12 dilution) using 250 mL and 1000 mL polypropylene graduated cylinders. Initial spectrophotometry results indicated that this protocol did not reliably produce a 5000 mg/L solution (data not shown). Therefore, in subsequent experiments, we adjusted the dilution to achieve a starting concentration of 5000 mg/L, as confirmed by spectrophotometry. We empirically determined that the starting concentration of the purchased bleach product was 4.5%, rather than the expected 6%. Thus, the final protocol used 111 mL of bleach product and 889 mL of tap water (1:9 dilution) to achieve a final concentration of 5000 mg/L.

Mimicking daily use of bottles. We sprayed bottles in Groups 1 and 2 (Figure 1) to mimic the effects of volume depletion and introduction of room air into spray bottles as a result of daily

Group	Bottle #	FAC Measuring Method	Storage Conditions
1	1	Colorimetric Chlorine Strips	Sprayed Daily
	2		
	3		
2	4	Spectrophotometry	
	5		
	6		
3	7	Colorimetric Chlorine Strips	Not Sprayed Daily
	8		
	9		
4	10	Spectrophotometry	
	11		
	12		

FAC, Free Available Chlorine

Figure 1. Experimental design by group, bottle identification, testing method, and storage conditions. The study design included 2 groupings of 6 bottles each: the first group (group 1 and 2) was sprayed daily to mimic use and the second group (group 3 and 4) was not sprayed, to test the role of air introduction in sodium hypochlorite degradation. Three bottles in each group (group 2 and 4) were monitored for free available chlorine (FAC) using spectrophotometry and three by colorimetric strips (group 1 and 3).

Figure 2. Set-up of the table and bottles within the empty rodent housing room. Twelve bottles (3 rows by 4 columns) were positioned at the center of a stainless-steel table, approximately 1 m tall, directly below the lighting banister. The exact positioning was adjusted until all bottles were exposed to the same average light intensity (lux), as confirmed by a light meter.

use. On days 0 to 42 of the experiment, we gently swirled each Group 1 and Group 2 bottle to thoroughly mix the solution and adjusted the nozzle until it produced a fine mist before continuing to spray for a total of 50 times per weekday based on our estimation of a standard workweek and the number of sprays required to saturate the working surface of an animal transfer station.

Sample collection for pH and FAC analysis. We sampled solutions weekly for the 6 wk duration of the experiment. We gently swirled bottles before twisting off the trigger spray. We transferred a 10 mL sample by a plastic serological pipette into a labeled 25 mL glass sample cell (Hach, Loveland, CO). We replaced the trigger spray and rinsed the serological pipette with tap water before proceeding to the next bottle. We collected sample cells into an enclosed cardboard box at room temperature until all samples were ready for analysis. We then measured the pH for all samples using an Orion 720A Plus digital pH meter (Fisher Scientific, Waltham, MA). The pH meter has a default resolution of 3 significant digits and an accuracy of ±0.002 pH. We calibrated the unit at the beginning of each time point using 3 standard solutions (pH 4, pH 7, pH 10). We adjusted the samples to a pH between 6 and 7 using 1N sulfuric acid before proceeding to FAC analysis.

Several methods can be used to measure and monitor FAC. The selection of specific methods depends on ease, precision and accuracy, available resources, and equipment. Semiquantitative methods include colorimetric strips that convert changes in FAC concentration to a visual color comparator. These strips are useful for nonregulatory reporting and for spot checking. Unfortunately, reading the color changes is subjective and is influenced by the light source and individual ability to judge subtle differences in color.[14] More accurate methods include spectrophotometry and iodometric titration. Spectrophotometry uses a photometer to accurately measure colorimetric changes that correspond with FAC concentrations. However, spectrophotometry is technically more challenging and requires equipment that ranges in price from a few hundred dollars to several thousand dollars.[14]

We measured the FAC concentrations in samples from Groups 2 and 4 using a DR2000 spectrophotometer (Hach, Loveland, CO) set to method 80 in accordance with USEPA DPD Method 8021.[3] This spectrophotometer has a resolution of 2 significant digits. Because we expected concentrations of FAC chlorine to exceed the upper range of the instrument (2.00 mg/L), the samples were diluted by transferring 4 µL of sample to a 10 mL glass sample cell (Hach, Loveland, CO) filled with deionized water. The diluted sample was then vortexed before proceeding with the remaining instructions for method 8021. A 1:2500 dilution factor was used to provide the highest resolution between expected readout values while minimizing error introduced by dilution. All samples were analyzed in triplicate.

FAC concentrations were estimated in samples from Groups 1 and 3 by using Bartovation extra high-level chlorine test strips (Queens, NY). These test strips detect FAC at the following concentrations: 0, 1000, 2500, 5000, 7500, 10000. One unblinded individual tested the samples following the manufacturer's protocol. An individual test strip was briefly submerged in the solution sample for one second, removed, and left on the benchtop for 30 s. The test strip color was evaluated within the next 10 s. All samples were analyzed in triplicate.

Statistical analysis. Statistical analysis was performed using Prism 9.1.2 (GraphPad Software, San Diego, CA). Group data were the mean ± 1 SD and individual data as the mean ± 1 SEM. Multiple unpaired *t* tests were used to compare group data for pH and FAC. Statistical significance was defined as a *P* value of less than 0.05 for all analyses.

Results

Environmental parameters. Environmental parameters measured in the experimental space (laboratory animal housing room) included room temperature, relative humidity, and light intensity (Figure 3A-C). Room temperature ranged from 18.9 to 21.1 °C, with a mean temperature of 21 °C (SD = 0.8) (Figure 3A). Relative humidity (as measured with a monitor placed at bottle height) ranged from 17% to 32%, with a mean of 20% (SD = 4) (Figure 3B). Light intensity (as measured with a lux meter centered over experimental bottles) ranged from 345 to 359 lx, with a mean of 352 lx (SD = 3) (Figure 3C). All environmental parameter measurements were within ranges commonly accepted as normal in animal housing units, with the exception of relative humidity, which was lower than usual in our experiment. However, relative humidity is commonly measured in animal spaces at the level of building ducts, rather than at the table height level measured here.

pH. pH values were generally stable in all bottles over the course of the experiment, despite slight decreases with time (Figure 4). Bottles in Groups 1 and 2 (Sprayed Daily) showed a mean baseline pH of 11.5 (SD = 0.05), which fell to 11.0 (SD = 0.06) by week 5 (Figure 4). pH values were not recorded for bottles in Groups 1 and 2 beyond week 5 because daily spraying completely depleted the bottles' contents. Bottles in Groups 3 and 4 (not sprayed daily) showed a mean baseline pH of 11.5

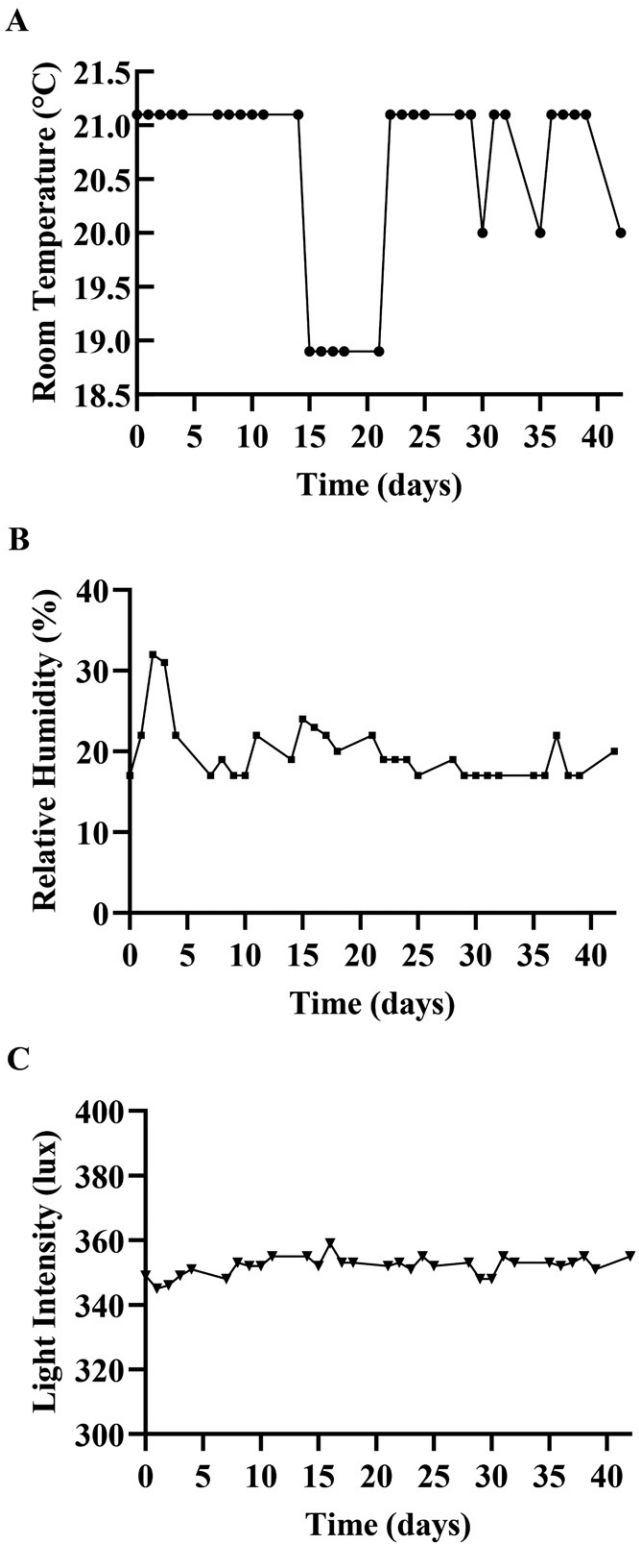

Figure 3. Environmental parameters recorded as (A) room temperature (°C), (B) relative humidity (%), and (C) light intensity (lux). The room temperature and relative humidity were measured using a thermo-humidity meter placed at the level of the bottles. (A) Room temperature remained relatively stable throughout the experiment except for a 7-d period where the temperature dropped below the lower limit (19.4 °C) to 18.9 °C. (B) Relative humidity varied between 17% and 32% throughout the experiment. (C) Light intensity was measured daily with a light meter and was very stable throughout the experiment with an average of 352 lx.

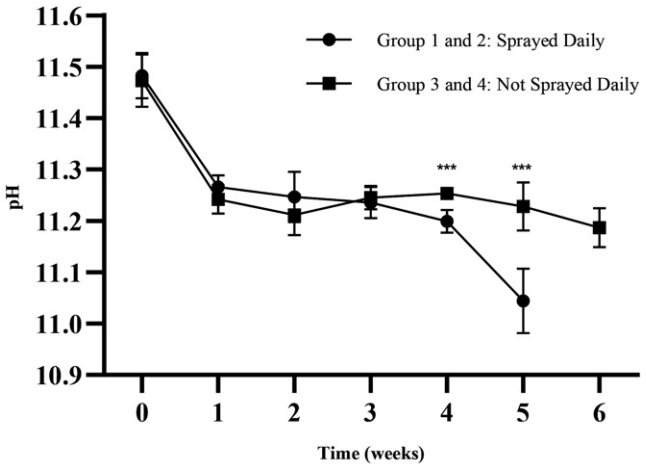

Figure 4. pH of solutions that were sprayed (Group 1 and 2) or not sprayed daily (Group 3 and 4) over time (mean ± 1 SD [error bars]). The pH values were generally stable throughout the experiment despite a slight decrease with time. Groups 1 and 2 (sprayed daily) had a baseline pH of 11.5 that decreased to 11.0 by week 5. Groups 3 and 4 (not sprayed daily) had a baseline pH of 11.5 that decreased to 11.2 by week 6. There was a statistically significant difference ($P < 0.05$) at weeks 4 and 5. Group 1 and 2 bottles (sprayed daily) had pH values of 11.2 and 11.0 compared with Group 3 and 4 bottles (not sprayed daily) that had pH values of 11.3 and 11.2 on weeks 4 and 5, respectively. Note that daily spraying completely depleted the bottle contents of Group 1 and 2 by week 5.

(SD = 0.05) that fell to 11.2 (SD = 0.04) by week 6 (Figure 4). Unpaired *t* tests comparing sprayed daily (combined data from Groups 1 and 2) and not sprayed daily (combined data from Groups 3 and 4) conditions showed a statistically significant difference ($P < 0.05$) at weeks 4 and 5, although effect sizes were small and unlikely to reflect functionally significant differences in disinfectant chemistry. Specifically, mean pH values for sprayed daily bottles were 11.2 and 11.0 for weeks 4 and 5, respectively. Mean pH values for unsprayed daily bottles were 11.3 and 11.2 for weeks 4 and 5, respectively.

Free available chlorine. Spectrophotometry measurements of FAC for 2 groups of bottles (Group 2 [sprayed daily] and Group 4 [not sprayed daily]) revealed that initial FAC levels were near the target starting concentration of 5000 mg/L; measured FAC concentrations were 4880 mg/L (SD = 23) in Group 2 and 4950 mg/L (SD = 12) in Group 4 (Figure 5C). FAC concentration fell slightly with time for all bottles, reaching a final concentration of 4600 mg/L (SD = 21) for Group 2 (at week 5) and 4630 mg/L (SD = 31) for Group 4 (at week 6) (Figure 5A-B). As noted above, Sprayed Daily bottles were empty by the end of week 5, due to daily spraying. Mean FAC loss from baseline levels was not significantly different between the 2 groups (6% for Group 2 at week 5 compared with 7% for Group 4 at week 6).

Colorimetric chlorine strips were used to evaluate FAC levels for the remaining 2 groups of bottles: Group 1 (sprayed daily) and Group 3 (not sprayed daily). All bottles in both groups showed concentrations at or near 5000 mg/L at all weekly time points throughout the experiment (5 wk for Group 1 and 6 wk for Group 2).

Discussion

This study evaluated an existing CDC guideline-based SOP for sodium hypochlorite solution preparation for laboratory animal use. The data showed that FAC levels remained stable for up to 6 wk. In addition, spray bottles in active use were de-

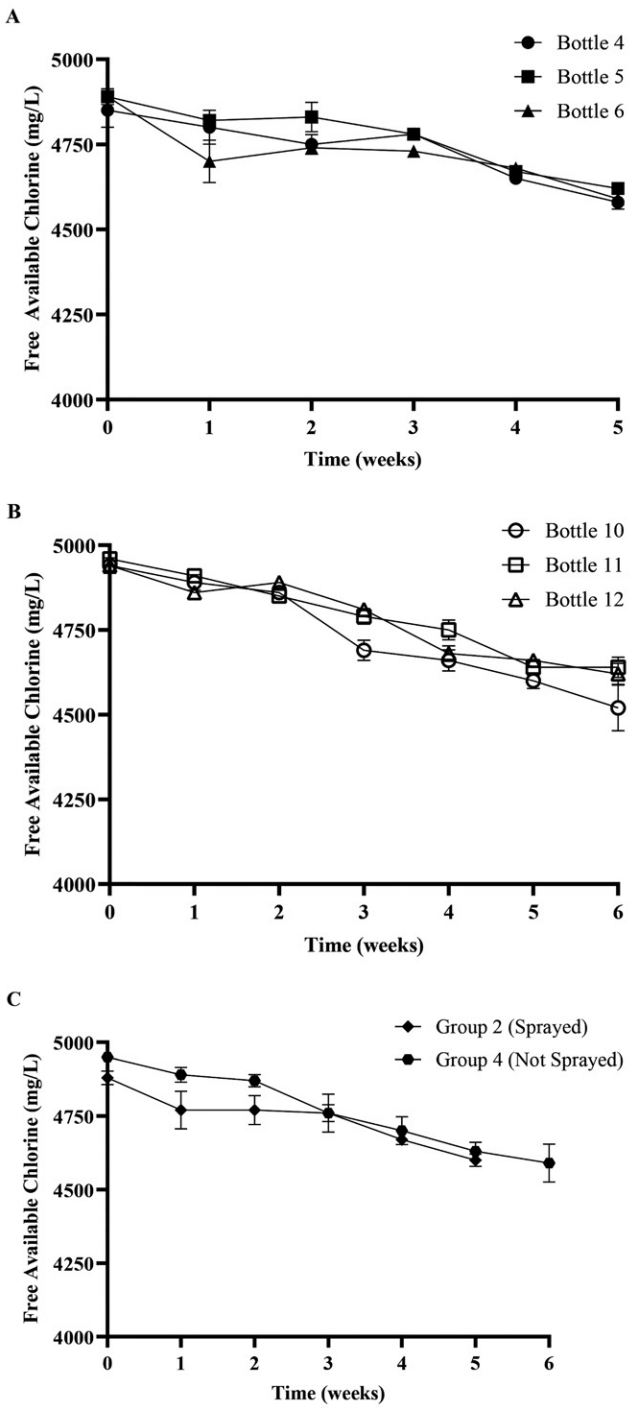

Figure 5. Degradation curves of dilute sodium hypochlorite solutions that were (A) Sprayed daily or (B) Not Sprayed Daily (mean ± 1 SEM [error bars]). (C) Group degradation curves (mean ± 1 SD [error bars]). Spectrophotometry measurements were taken for Group 2 and Group 4 bottles. (A) This depicts the individual degradation curves for bottles 4, 5, and 6 (Group 2) over 5 wk. All time points were performed in triplicate. (B) This depicts the individual degradation curves for bottles 10, 11, and 12 (Group 4) over 6 wk. All time points were performed in triplicate. (C) Initial FAC levels for Group 2 were 4880 mg/L (SD = 23) and 4950 mg/L (SD = 12) for Group 4. FAC concentration decreased slightly with time for all bottles, to a final concentration of 4600 mg/L (SD = 21) for Group 2 (at week 5) and 4630 mg/L (SD = 31) for Group 4 (at week 6). Mean FAC loss from baseline levels was slightly higher in the not sprayed daily (Group 4) bottles (6% for Group 2 at week 5 compared with 7% for Group 4 at week 6), although this difference was not statistically significant. Note that daily spraying completely depleted the bottle contents of Group 2 by week 5.

pleted of diluted solution by 5 wk, suggesting that bottles can be refilled with fresh solution when empty without any loss of disinfection effectiveness in practice. This finding showed that laboratory animal programs can reduce labor and reagent costs associated with daily sodium hypochlorite solution preparation without compromising animal or technician safety. Our data also showed that actual concentrations of commercial bleach concentrates may be significantly lower than what is reported on consumer labels. This was also noted in another study that reported variability in advertised and measured concentrations of commercial bleach samples from different countries.[12] These findings strongly suggest that laboratory animal programs should empirically test the starting concentrations of purchased commercial bleach brands to develop dilution protocols that yield the desired 5000 mg/L disinfection concentration for use in animal spaces.

Prior studies show that extrinsic factors, including light, temperature, and air in bottle headspace, are important predictors of sodium hypochlorite solution stability.[1,2,5,6,8-13,15-17] We found that these factors remain reasonably stable in OHSU DCM animal housing spaces. We acknowledge that natural fluctuations in environmental parameters can be expected in any representative animal care and use program. Temperature was largely stable, apart from a 7-d period during which room temperature dropped to 18.9 °C, a 0.5 °C dip from the room minimum, possibly due to the absence of major thermal output (active racks with live animals) in the study room. Relative humidity levels, while not previously reported to affect FAC degradation, were out of the intended range for the majority of our experiment. We tracked temperature and relative humidity by using a thermo-humidity meter at the level of the bottles. These measures are highly dependent on placement of the meter within the room. Furthermore, relative humidity in animal rooms is commonly measured at the level of the building ducts. Light intensity, reported as lux, was within the range prescribed by the *Guide for the Care and Use of Laboratory Animals*, which recommend that empty rooms not exceed 400 lx approximately 1 m from the ground.[7] Conditions in this experiment represented the most extreme light conditions likely in an animal housing room. Prior studies found that light quality and quantity influence bleach stability.[2,5,8,9,11,16,17] Most, if not all, rodent housing rooms are completely devoid of natural light, so the compact fluorescent light tested in this experiment is most relevant to practice. Compact fluorescent bulbs emit a small amount of UVA, UVB, and infrared radiation, which we hypothesized might speed FAC degradation.[19] However, we found minimal decreases in FAC over the course of the study, such that environmental parameters reported here did not appreciably affect FAC concentrations. These FAC results may not be generalizable to laboratory animal spaces with less tightly controlled environmental parameters.

Prior studies also found that intrinsic factors, including pH and baseline FAC concentrations, contribute to bleach stability. Commercial bleach is commonly manufactured to a final pH greater than 11 because sodium hypochlorite solutions are more stable at higher pH.[5,16] Dilution of sodium hypochlorite with water lowers pH, which may speed solution degradation. Degradation increases at pH levels between 11 and 7 and increases precipitously at pH < 7.[1,5] Here, we found that the current OHSU DCM protocol yields a dilute sodium hypochlorite solution of pH approximately 11, and that this pH was relatively stable throughout the experiment, indicating that common practice and conditions will produce and maintain solutions of reasonable stability. Initial FAC concentrations can also affect solution

degradation. Solutions with relatively higher initial FAC concentrations generally degrade more quickly than solutions with lower starting FAC.[2,8,17] We found that the OHSU DCM protocol (adjusted for empirically measured commercial bleach concentration) yields dilute bleach solutions that vary minimally in starting FAC concentrations and that this minimal variance has negligible effects on solution degradation rate.

Measurement of FAC by spectrophotometry may not be feasible for all laboratory animal programs. Therefore, we tested the effectiveness of inexpensive and readily available colorimetric strips for evaluation of FAC concentrations. Colorimetric strips have lower resolution than spectrophotometry and can therefore not detect small fluctuations in FAC concentration. However, in this study, we found that fluctuations in FAC in dilute sodium hypochlorite solutions over 5 to 6 wk were not detectable on colorimetric strips; all bottles showed concentrations at 5000 mg/L, as measured by the strips. However, the FAC fluctuations that occurred in this study were minor, and this experiment spanned the full length of the likely "lifespan" of a bottle of solution in regular use, suggesting that colorimetric strips are a useful tool for a quick determination of adequate disinfection capacity of a given dilute solution. These strips may be a useful quality control tool for detecting the FAC of freshly prepared solutions, manufacturing changes in starting bleach concentrations, and errors in dilution. These events are not unlikely, as evidenced by the lower-than-expected starting concentration in the commercial bleach product used in this experiment. As mentioned in the materials and methods section, we altered the dilution equation from our SOP after determining that the FAC of the stock bleach bottle was lower than that stated on the bottle. Typically, the consumer does not know the lag in time between creation of the sodium hypochlorite solution and its distribution and use; increased distribution lag times may contribute to lower stock bleach concentrations. The CDC guidelines for disinfection and sterilization in healthcare settings and the instructions given by the bleach manufacturer recommend testing the solution using a quantitative method to fine tune dilutions to the specific concentration required.[12,18] However, the importance of using a 5000 mg/L sodium hypochlorite concentration may depend on disinfection use. For example, OHSU DCM's working solution (0.5%) is a much higher concentration than that required to kill a large majority of the microorganisms listed in the CDC guidelines (Table 1). This provides room for error. If a laboratory animal program has concerns about a specific microorganism that requires a high concentration of sodium hypochlorite, they may opt to use a higher concentration, or, alternatively, they may choose a different disinfection protocol that is more powerful (that is, vaporized hydrogen peroxide, chlorine dioxide).

Table 1. Microbicidal activity of chlorine and chlorine compounds. The table, according to the CDC guidelines on disinfection, lists the FAC concentration and contact time required to render the listed microorganisms inactive.

Microorganism	FAC Concentration Required for Disinfection (ppm)	Contact Time
Mycoplasma	25	15 s
Vegetative bacteria	<5	30 s
Mycobacterium tuberculosis	1000	<10 min
Bacillus atrophaeus spores	100	5 min
Mycotic agents	100	<1 h
Clostridium difficile	5000	≤10 min
25 different viruses	200	10 min
Candida	500	30 s
Staphyloccous aureus	100	<10 min
Salmonella cholerasuis	100	<10 min
Pseudomona aeruginosa	100	<10 min

FAC, Free Available Chlorine
ppm, parts per million

This experiment has several limitations. First, our results may be limited by our small sample size. We tested only 3 bottles per group, which is significantly fewer than the number of bottles in use in OHSU DCM. However, our sample size was consistent with other studies evaluating bleach stability, and we tested each bottle in triplicate to increase precision.[10,11] Second, the colorimetric strips we used have poor resolution at high concentrations, and we were unable to find alternative strips with better resolution. Manufacturers produce most colorimetric strips for FAC level estimation to determine the safety of drinking water treated with low levels of chlorine; they produce few colorimetric strips for use in high concentration solutions. In this experiment, all bottles tested with colorimetric strips were at the 5000 mg/L mark during the entire length of the experiment. However, these strips would not record less than a 50% loss in FAC; the next "color bar" records 2500 mg/L FAC. Therefore, these strips would only detect large errors or changes in manufacturing protocol. The colorimetric strips were also read by one unblinded individual. Results could have been strengthened by blinding the reader to the bottle conditions. However, this study was performed during the COVID-19 pandemic under modified operations at OHSU. Modified operations and campus research requirements dictated that only one individual at a time could work in a small, enclosed space. Third, we only tested 1 starting concentration of a dilute sodium hypochlorite solution (0.5% or 5000 mg/L) generated from a single commercial brand of bleach. Care should be taken when extrapolating to other bleach brands without empirical testing of FAC concentrations because starting concentration can influence bleach degradation. Fourth, we did not test for efficacy of solution disinfection through methods recognized by the Association of Official Analytical Chemists against common microorganisms; instead, we relied on published sodium hypochlorite concentrations previously reported to be effective. Last, we recognize that daily use in this study was an estimated average and that factors such as variability in how much people spray to clean surfaces and in how rooms are used contributes to how quickly bottles are emptied. Consideration for average room usage across facilities and animal care technician room task schedules will determine the exact rate at which sodium hypochlorite solutions are replaced; our data indicate that bottles can be prepared weekly, every other week, or possibly monthly. The findings of our study also support the need for a resource that shares detailed "true use" protocols for disinfectants to promote uniformity in protection.

The goal of this project was to provide practical guidance for the DCM on bleach stability. Here we show that in real-world conditions, 0.5% sodium hypochlorite solutions stored and used in a standard rodent housing room in opaque plastic bottles sprayed daily will maintain acceptable FAC concentrations for 5 to 6 wk, perhaps longer. Our results should directly inform practice changes that reduce technician time in preparing sodium hypochlorite bottles daily, reduce waste and help conserve resources, and reduce overall facility costs.

Acknowledgments

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References

1. **Camps J, Pommel L, Aubut V, Verhille B, Satoshi F, Lascola B, About I.** 2009. Shelf life, dissolving action, and antibacterial activity of a neutralized 2.5% sodium hypochlorite solution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **108:**e66–e73. https://doi.org/10.1016/j.tripleo.2009.03.034.
2. **Clarkson RM, Moule AJ, Podlich HM.** 2001. The shelf–life of sodium hypochlorite irrigating solutions. Aust Dent J **46:**269–276. https://doi.org/10.1111/j.1834-7819.2001.tb00291.x.
3. **Eaton AD, Clesceri LS, Greenberg AE, Franson MAH.** 2017. American Public Health Association, American Water Works Association, and Water Environment Federation. 4500–Cl–CHLORIDE. In: Standard methods for the examination of water and wastewater, 23rd ed. Washington (DC): American Public Health Association.
4. **Farr JP, Smith WL, Steichen DS**. Bleaching agents (survey), p. 271–300. In: Kirk–Othmer encyclopedia of chemical technology. New York (NY): Chichester Wiley.
5. **Frais S, Ng YL, Gulabivala K.** 2008. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite. Int Endod J **34:**206–215. https://doi.org/10.1046/j.1365-2591.2001.00371.x.
6. **Gallandat K, Kolus RC, Julian TR, Lantagne DS.** 2021. A systematic review of chlorine–based surface disinfection efficacy to inform recommendations for low–resource outbreak settings. Am J Infect Control **49:**90–103. https://doi.org/10.1016/j.ajic.2020.05.014.
7. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals: 8th ed. Washington (DC): The National Academies Press.
8. **Iqbal Q, Lubeck–Schricker M, Wells E, Wolfe MK, Lantagne D.** 2016. Shelf–life of chlorine solutions recommended in ebola virus disease response. PLoS One **11:**e0156136. https://doi.org/10.1371/journal.pone.0156136.
9. **Ishihara M, Murakami K, Fukuda K, Nakamura S, Kuwabara M, Hattori H, Fujita M, Kiyosawa T, Yokoe H.** 2017. Stability of weakly acidic hypochlorous acid solution with microbicidal activity. Biocontrol Sci **22:**223–227. https://doi.org/10.4265/bio.22.223.
10. **Johnson BR, Remeikis NA.** 1993. Effective shelf–life of prepared sodium hypochlorite solution. J Endod **19:**40–43. https://doi.org/10.1016/S0099-2399(06)81040-X.
11. **Kobylarz J.** 2017. Efficacy of chlorine sanitizer spray bottles exposed to various temperatures and ambient light. BCIT Environmental Public Health Journal. https://doi.org/10.47339/ephj.2017.74
12. **Lantagne DS.** 2009. Viability of commercially available bleach for water treatment in developing countries. Am J Public Health **99:**1975–1978. https://doi.org/10.2105/AJPH.2009.160077.
13. **Lantagne PED, Preston K, Blanton E, Kotlarz N, Gezagehn H, van Dusen E, Berens J, Jellison K.** 2011. Hypochlorite solution expiration and stability in household water treatment in developing countries. J Environ Eng **137:**131–136. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000299.
14. **Murray A, Lantagne D.** 2015. Accuracy, precision, usability, and cost of free chlorine residual testing methods. J Water Health **13:**79–90. https://doi.org/10.2166/wh.2014.195.
15. **Nicoletti MA, Siqueira EL, Bombana AC, Oliveira GG.** 2009. Shelf–life of a 2.5% sodium hypochlorite solution as determined by Arrhenius equation. Braz Dent J **20:**27–31. https://doi.org/10.1590/S0103-64402009000100004.
16. **Piskin B, Turkun M.** 1995. Stability of various sodium hypochlorite solutions. J Endod **21:**253–255. https://doi.org/10.1016/S0099-2399(06)80991-X.
17. **Rutala WA, Cole EC, Thomann CA, Weber DJ.** 1998. Stability and bactericidal activity of chlorine solutions. Infect Control Hosp Epidemiol **19:**323–327. https://doi.org/10.2307/30141372.
18. **Rutala WA, Weber DJ.** 2008. Guideline for disinfection and sterilization in healthcare facilities. Washington (DC): Center for Disease Control
19. **United State Food and Drug Administration.** [Internet]. 2017. Compact fluorescent lamps (CFLs) – Fact Sheet/FAQ. [Cited 1 July 2021]. Available at: https://www.fda.gov/radiation–emitting–products/home–business–and–entertainment–products/compact–fluorescent–lamps–cfls–fact–sheetfaq#:~:text=into%20this%20area.–,Do%20CFLs%20emit%20UV%3F,measuring%20equipment%20must%20be%20used.