ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

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INACTIVATION OF BIOHAZARDOUS MATERIALS FOR FUTURE USE

Introduction

By UNL policy, biohazardous materials must be decontaminated prior to removal from a lab unless being shipped following IATA/DOT packaging rules or they are safely contained and being moved to an equivalent containment space (e.g., BSL-2 lab to BSL-2 lab). Refer to the EHS SOPs ***Disposing of Biohazardous Materials***, ***Shipping of Biological Substances and Patient Specimens***, and ***Transport of Biohazardous Materials at UNL*** for guidance on disposal, shipping and transport of biohazardous materials.

However, sometimes it is necessary to move biohazardous materials to spaces with lower containment levels to perform further analysis or utilize certain pieces of equipment. To do this safely, the material must be inactivated to render the material non-viable, non-infectious and/or non-toxic while maintaining characteristics of interest. Decontamination and disinfection processes often remove or damage characteristics of interest in the material (e.g., DNA/RNA integrity for PCR; peptides, proteins, and antibodies for ELISA, etc.). This document provides guidance about the selection, validation, and testing of procedures for inactivation of biohazardous materials for future use. The guidance provided in this document is derived from nationally recognized standards and should be considered when biohazardous materials are moved to lower containment.

Definitions:

Decontamination: The use of physical and/or chemical means to remove, inactivate, or destroy microbial pathogens (e.g. bloodborne or aerosolized) on a surface or item to the point where they are no longer capable of transmitting infectious particles and the item or surface is rendered safe to handle.

Disinfection: A process that destroys pathogens and other microorganisms, except prions, by physical or chemical means.

Inactivation: A procedure to render a pathogen non-viable, viral nucleic acid sequences non-infectious, or a toxin non-toxic while retaining characteristic(s) of interest for future use. Methods targeting tropism may be host-specific.

Scope

- **For BSL-3/ABSL-3 labs:** All inactivation procedures **must** be approved by the IBC and outlined in the IBC protocol. Deviation from approved procedures is not permitted without prior approval. Use of a validated[1] procedure with *in-house* verification is **required** when transferring samples from any high-containment (ABSL-3, BSL-3) laboratory at UNL to another campus facility of lower containment.
- **For BSL-2/ABSL-2 labs:** Use of validated[1] inactivation procedures and in-house verification of inactivation procedures is highly recommended and inactivation procedures should be included in the IBC protocol. Biohazardous materials removed from a BSL-2 laboratory which have ***not*** been inactivated should be accompanied by a document indicating such (see example in Appendix A below), along with affixing the universal biohazard symbol on the container(s)

IMPORTANT: Any inactivation of Select Agents for subsequent transfer must be validated and documented according to Federal Select Agent Program regulations. Please contact the UNL Biosafety Officer for additional information and guidance.

Inactivation methods

When choosing an inactivation method, several factors need to be considered including specific controls; the balance between efficacy of inactivation vs. the retention of desired characteristics; and the appropriate safety margin (i.e., overkill amount). Additional advantages may include low cost and broad applicability to different types of agents.

The starting point for development of an inactivation procedure is deciding which inactivation method(s) is appropriate, effective, and feasible to use for the desired outcome and use of the material. Validated inactivation procedures considered can be based on:

1. A procedure developed in-house;
2. A procedure published in a peer-reviewed journal; or
3. A commonly accepted method (e.g., heat, dry or wet).

Many variables need to be considered when developing inactivation protocols, which include:

- Volume of agent;
- Nature of agent (nucleic acid, pathogen, toxin);

[1] Validated means proven to be effective through repetition in-house or from published peer-review articles or other trusted sources.

- Matrix/solvent surrounding the agent;
- Type of container used;
- Safety measures needed (PPE, waste disposal, etc.)

Surrogate strains or agents may be used when limited samples are available to develop the inactivation procedures. Suitable surrogates would be bacteria from the same genus and viruses from the same family. Tissue surrogates may be appropriate in some situations. For example, tissue adjacent to the tissue of interest that has also undergone the inactivation may be used for confirmation of the inactivation procedure and verification that adequate efficacy has been achieved in the process.

Listed below are general categories of inactivation which may be considered (not an exhaustive list). Researchers are encouraged to consult peer-reviewed, scientific publications for additional information on agent-specific inactivation methods which may be employed and validated *in-house*.

A. Physical Inactivation. This process employs heating or irradiating the samples, to predetermined parameters, resulting in membrane disruption and/or protein and nucleic acid denaturation. It is important to ensure the samples are exposed for the time required for inactivation. Examples of physical inactivation methods include:

- Heating samples to 56 ˚C or higher for specified time exposure.
- Exposing samples to UV-C or other ionizing irradiation.

B. Natural antimicrobial strategies. Use of antimicrobial enzymes or peptides have been effective strategies for inactivation. Parameters will vary according to specific needs and agents in use. Examples of antimicrobial compounds include:

- Lysozyme;
- Antimicrobial peptides.

C. Chemical Inactivation. This process employs the use of denaturing chemicals (alcohols, aldehydes, detergents) to denature proteins and render the agent(s) inactive. Concentration and exposure time of denaturing chemicals varies depending on the type of agent (virus, bacteria, fungi, or toxin) manipulated. Examples of chemicals used for inactivation include:

- Glutaraldehyde, Formaldehyde, Paraformaldehyde;
- Triton X-100; Sodium Dodecyl Sulfate;
- Phenol/Chloroform/Isoamyl alcohol;
- Ethanol, Diethyl ether, Acetone

D. **Combination of the above methods.** It is possible to combine the strategies listed above to achieve complete inactivation (e.g. heating samples followed by chemical inactivation).

Validation of Inactivation Procedures

Inactivation procedures must be optimized for efficacy and modified for the specific materials and circumstances present in each setting. A validated inactivation procedure will designate a set of conditions that have been verified to adequately render:

- A pathogen non-viable, with efficacy established by viability testing data;
- The isolated viral nucleic acid incapable of producing infectious forms of virus, with efficacy established by infectivity testing data; or
- A toxin no longer capable of exerting a toxic effect, with efficacy established by toxicity testing data.

Inactivation procedures must be validated through a viability testing protocol. This is a process to confirm efficacy of the inactivation procedure by demonstrating the material is free of all viable pathogens. Methods of testing include:

- Cell viability (viruses, infectious nucleic acids);
- Growth assays (bacteria);
- Toxin functional activity and *in-vivo* exposure.

Process verification

Inactivation methods must be appropriately verified in the hands of the researcher(s) performing the procedure while using the reagent sources and equipment intended for the routine process. Verification must occur regardless of the source of the procedure as there can be variability in reagents, equipment and environmental conditions that can impact the outcome of the inactivation process.

The potential for incomplete inactivation, including errors that might result from exceeding the capacity of the inactivating process to kill the pathogen, lack of specificity, detection limits, and run-to-run variation should be considered when setting specifications for confirmed inactivation procedures.

Verification of inactivation processes should be replicated sufficiently ($n \geq 2$) to determine the underlying variability within the procedure in the hands of the person(s) performing it. Other considerations for inactivation procedure development include:

- Any chemical inactivation treatments that need to be neutralized or diluted prior to the confirmation testing; and

- The statistical probability of inactivation (i.e., was the sample subject to sufficient inactivating material/process to provide a statistically significant probability of complete inactivation).
- Incomplete inactivation of the sample increases the risk of accidental release or exposure.

FDA-approved sample collection kits with integrated pathogen inactivation are considered verified and can be used without *in-house* process verification.

Documentation and Recordkeeping

Documentation of validated inactivation procedures should be kept in the laboratory biosafety manual and updated as needed. These **records are required** for BSL-3 labs and are recommended at BSL-2. Information recorded should include:

- Name of person conducting the inactivation;
- Procedure(s) employed;
- Date and location of inactivation;
- Identity of agent(s), including any genetic modifications;
- Results of the inactivation (enumeration of growth, plaque assay results, etc.),

These records should be shared with sample recipients when material is sent to other institutions.

Routine verification of inactivation procedure(s)

As procedures and experiments evolve, including new genetic recombination experiments, it is necessary to regularly verify that any inactivation procedure used remains valid, with procedural adjustments made as needed. Re-verification of inactivation procedures should be performed and documented for:

- New strains/pathovars within the same species of pathogenic microorganism;
- New or altered virulence genes or factors;
- Altered host range of pathogen or toxin.

It should ***never*** be assumed that a verified inactivation procedure is valid for any altered pathogens or toxins, regardless of modification. It is the responsibility of the PI to ensure inactivation procedures are routinely verified and the results documented in the laboratory biosafety manual.

IMPORTANT: Any accidental release or exposure to a pathogen or toxin as a result of failed inactivation may result in a report sent to the applicable regulatory authority (NIH, CDC, USDA, etc.), not to mention the possibility of an exposure incident being reported by the news media and damaging the reputation of the PI and UNL.

References

1. *Appendix K*, **Biosafety in Microbiological and Biomedical Laboratories** (BMBL; 6th ed.). Center for Disease Control and Prevention, National Institutes of Health, 2020
2. **Laboratory Biosafety Manual**, 4th ed., World Health Organization, 2020
3. **Decontamination and waste management monograph**. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)

Appendix A: Sample form for biohazardous materials which have not been inactivated. This form is <u>not</u> to be used for agents requiring high-containment (ABSL-3; BSL-3) facilities.

Warning: Biohazard Materials, Potentially Infectious

CAUTION: Items in this container have **not** been inactivated and may be infectious. Handle using appropriate biosafety containment procedures.

Agent (genus, species)	
Pathogenicity (human, animal, plant)	
Biosafety Containment Level	
Mode of Transmission	
Genetic modifications	
Emergency Contact information	