Tarrant County Public Health
1101 South Main Street
Fort Worth, TX 76104

INACTIVATION OF SELECT AGENTS

Approved by	
Date approved	
Revised by	Erin Taylor
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I. INTRODUCTION

This policy/procedure provides the general/basic biosafety requirements to be followed in order to safely and effectively work with materials derived from infectious select agents. Further, the established and validated inactivation procedure is specified. There are no plans of sharing or shipping any of the materials derived from select agents at this registered facility, North Texas Regional Laboratory (NTRL), Tarrant County Public Health. Inactivated materials are only used for diagnostic and environmental testing.

II. PURPOSE

To ensure compliance and adherence with select agent regulations (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73). The emphasis is to describe how materials are processed and assessed before any work with inactivated materials is conducted outside of BSL-3.

III. EXAMPLES OF SELECT AGENTS

The same procedure must be followed whether materials are *known* to contain a select agent, or if materials are tested for ruling-out *any* select agents. Here is a sample list of bacterial, infectious select agents that our laboratory could potentially encounter:

***B. anthracis*:**	gram-positive, spore-forming bacteria; durable to heat and desiccation.
B. cereus:	*Bacillus cereus* biovar *anthracis;* gram-positive, spore-forming bacteria; durable to heat and desiccation.
Burkholderia:	*Burkholderia mallei* and *B. pseudomallei;* non-spore-forming, gram-negative bacteria.
***F. tularensis*:**	non-spore-forming, gram-negative bacteria.
***Y. pestis*:**	non-spore-forming, gram-negative bacteria.

IV. TYPES OF MATERIALS DERIVED FROM SELECT AGENTS

The main concern for our laboratory (NTRL) is nucleic acid extracts derived from select agents. In order to carry out Polymerase Chain Reaction (PCR) analysis, the nucleic acid needs to be transported from BSL-3 space to BSL-2 space, where the PCR instruments are located. Currently, there are three types of extraction methods used in BSL-3, namely:

a) Cell lysate prepared from an isolate actively growing on an agar plate.
b) Qiagen EZ1 Advanced XL automated extraction, which is mainly used for extraction of environmental samples for screening for multiple agents.
c) Manual Qiagen QIAmp DNA Blood Mini Kit extraction, which is used for direct clinical specimen extractions.

V. INACTIVATION PROCEDURE

All of the extraction methods are in themselves, to varying degrees, contributing to inactivation of select agents. More importantly, independently of extraction method, all extracts are filtered through a 0.1 µm centrifugal filter unit before removal from BSL-3. Filtration allows the removal of any viable cells (vegetative and spores) potentially present in the extracts. 10% of the filtered eluate is spread on an agar plate, which is incubated at 35-37 °C and observed for a minimum of five days to confirm sterility. The results of sterility testing to date have been summarized in a separate sterility testing validation study.

Step-by-step procedure for inactivation and sterility verification:

1. Prepare a nucleic acid extract in BSL-3, using any of the extraction methods listed in Section IV.
2. Filter the entire volume of the nucleic acid extract by using a 0.1 µm centrifugal filter unit.
3. Select an appropriate agar plate for sterility testing. In general, a 5% Sheep's Blood Agar (SBA) plate is used. However, if the extract is made from *Francisella tularensis*, a Chocolate (CHOC) plate must be used instead of SBA.
4. Withdraw a minimum of 10% of the extract and spread on an agar plate. Examples:
    a. For a 100 µl eluate, spread at least 10 µl on the appropriate agar plate.
    b. For a 200 µl eluate, spread at least 20 µl on the appropriate agar plate.
5. Incubate the agar plate at 35-37 °C for a minimum of 5 days and make notes of any growth and absence of growth. Make sure to make notes of any suspected environmental contaminant as well. The person doing the final read for sterility must indicate the number of days the plate was incubated, then sign and date the final interpretation.
6. Things to consider when interpreting the results:
    a. Occasionally, environmental contaminants may grow on the sterility plate. It is important to determine if the growth resembles any of the select agents listed in Section III. If that is the case, further characterization is necessary, which may include both culture methods and PCR to rule-out that a select agent is present in the filtered eluate.
    b. If it is determined that a select agent is growing on the sterility plate, immediately notify the Responsible Official (RO) or an Alternate Responsible Official (ARO). The potential occupational exposure must be documented and reported using the APHIS/CDC Form 3. The RO or ARO will determine what other actions may be necessary.
7. As soon as the extract has been filtered and a sample has been spread on the sterility plate, the extract may be removed from BSL-3 and used for PCR. It is important to remember that any potential select agent will be inactivated and removed through the combination of extraction and filtration, and sterility testing is just a way to confirm that the process was performed appropriately.

VI. SHIPPING OF SELECT AGENTS OR INACTIVATED MATERIALS

NTRL personnel are trained in shipping Category A select agents. Inactivated material derived from select agents has never been shipped, and there is no intention to do so. If a need to ship inactivated material would arise, and if the materials were to be derived from *B. anthracis* or *B. cereus* biovar *anthracis*, we would utilize the procedure outlined in the policy document "Inactivation of *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis"* (see references), which involves using a liquid broth. However, testing at NTRL is done for the purpose of identifying or ruling out select agents in diagnostic and environmental samples. Research or sharing of materials outside the laboratory is not done. The only exception would be if materials need to be shipped to the Centers for Disease Control and Prevention (CDC), but that would involve using an APHIS/CDC Form 2 for transfer of viable select agent.

VII. BIOHAZARDS RISK MITIGATION AND TRAINING

All personnel working with select agents must have passed a Security Risk Assessment (SRA) and been trained in all pertinent Laboratory Response Network (LRN) procedures, and laboratory-specific Standard Operating Procedures (SOPs) related to select agents. Key areas addressed during training include Personal Protective Equipment (PPE), working in BSL-3, proper use of a Biosafety Cabinet (BSC), autoclaving of biohazardous waste, decontamination and spill procedures, select agent inventory/usage logs, and agent-specific risks. In particular, preparation of nucleic acid extracts, sterile filtration, and verification of sterility is part of the hands-on training all personnel must complete before working without direct supervision.

VIII. REFERENCES

- CDC Policy statements pertinent to this Standard Operating Procedure:
  - Validation of Inactivation Procedures (April 6, 2017): https://www.selectagents.gov/policystatement_inactivation.html
  - Inactivation of *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* (April 19, 2017): https://www.selectagents.gov/policystatement_bacillus.html
- APHIS/CDC Form 2:
  - Request to transfer select agents/toxins: https://www.selectagents.gov/form2.html
- APHIS/CDC Form 3:
  - Incident form for reporting potential theft, loss, release, or occupational exposure: https://www.selectagents.gov/form3.html
- Sterility testing validation study:
  - NTRL validation write-up on sterile-filtered extracts obtained through cell lysate preparation, MagNA Pure Compact extraction, or Qiagen extraction.
- Laboratory Response Network (LRN) extraction procedures:
  - Cell lysates: Document #: LRN-1103
  - Qiagen EZ1 Advanced XL: Document #: LRN-1214
  - Qiagen QIAmp DNA Blood Mini Kit: Document #: LRN-1018
- List of agents excluded from the inactivation regulatory provisions may be found at https://www.selectagents.gov/SelectAgentsandToxinsExclusions.html.