Guidance on Non-viable Select Agents and Nonfunctional Select Toxins and Rendering Samples Free of Select Agents and Toxins

Federal Select Agent Progam

Animal and Plant Health Inspection Service/Agriculture Select Agent Services

Centers for Disease Control and Prevention/Division of Select Agents and Toxins

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Preface

The information in this guidance document is meant to provide additional information to regulated entities to assist them in meeting the requirements for select toxins.

Revisions: This is a living document subject to ongoing improvement. Feedback or suggestions for improvement from registered Select Agent entities or the public are welcomed. Submit comments directly to the Federal Select Agent Program at:

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Contents

Introduction 4

Terminology 5

Inactivation 5

Viability Testing 6

Neutralization 6

Scenarios 7

DRAFT

Introduction

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulatory requirements for the possession, use, and transfer of biological agents and toxins that have the potential to pose a severe threat to public health and safety, animal and plant health, and animal and plant products. These requirements can be found at 42 CFR Part 73 (HHS), 7 CFR Part 331 (USDA-Plant Protection and Quarantine), and 9 CFR Part 121 (USDA-Veterinary Services) and are referred to as the select agent regulations (SAR).

The SAR state that non-viable select agents or nonfunctional select toxins are excluded from the requirements of the SAR [See 7 CFR §331.3(d)(2); 9 CFR §121.3(d)(2) and 9 CFR §121.4(d)(2); 42 CFR §73.3(d)(2) and 42 CFR §73.4(d)(2)]. Since the implementation of the SAR, there have been several events related to select agents that were presumed to be non-viable. For example,

- a shipment of presumed non-viable select agent material that subsequently resulted in the handling of viable select agents in unregistered entities using inappropriate containment;
- the commingling of presumed non-viable select agent inventory, subsequently determined to be viable, with non-select agent inventory resulting in the use of insufficient laboratory safeguards.

This guidance does not apply to:

- Products that are, bear, or contain listed select agents or toxins that are cleared, approved, licensed, or registered under the provisions of 7 CFR §331.5(c), 9 CFR §§121.5(c), 121.6(c), 42 CFR §§73.5(c), 73.6(c)[1], insofar as their use meets the requirements of such laws
- select agents and toxins excluded as an attenuated strain of a select agent or a select toxin modified to be less potent or toxic under the provisions of 7 CFR §331.3(e), 9 CFR §§121.3(e), 121.4(e), 42 CFR §§73.3(e), 73.4(e) [2]
- inactivation for waste disposal (More information on the use of infectious waste treatment can be found at http://www.selectagents.gov/guidance-wastetreatment.html)

This guidance does apply to the future use of select agents or toxins that have undergone an inactivation process and are excluded as nonviable or nonfunctional under the provisions of 7 CFR§§331.3(d)(2), , 9 CFR§§121.3(d)(2), 121.4(d)(2), 42 CFR§§73.3(d)(2), 73.4(d)(2).

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[1] This guidance does not apply to products that are, bear, or contain listed select agents or toxins that are cleared, approved, licensed, or registered under the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et seq.), section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C. § 262), the Virus-Serum-Toxin Act (21 U.S.C. §§ 151-159), or the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 136 et seq.).

[2] This guidance does not apply to specific attenuated select agents or a select toxin modified to be less potent or toxin approved by the FSAP so long as the attenuated strain or modified toxin is not subjected to any manipulation that restores or enhances its virulence or toxic activity. Attenuations are usually specific manipulations targeted at the genomic level to alter gene expression rendering a select agent less virulent or avirulent, or a toxin less toxic; however, the agent or toxin usually remains viable or functional to some extent (e.g., the knockout of an essential virulence gene). Please refer to the "Exclusion Guidance Document" and exclusion list available at www.select agents.gov for additional information.

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Terminology

For the purpose of the SAR, "non-viable" and "nonfunctional" are similar terms that may be read as referring to the loss of biological activity. For a select agent, the term "non-viable" means that a select agent is no longer capable of growing, replicating, infecting, or causing disease. For regulated nucleic acids, the term "non-viable" means that the nucleic acids are no longer capable of producing infectious forms of a select virus or expression of a functional select toxin without further genetic manipulation. For a select toxin, the term "nonfunctional" means a toxin is no longer capable of exerting a toxic effect. As used in this guidance, the term "inactivation" is defined as "a method to render a select agent or regulated nucleic acids as non-viable or a select toxin nonfunctional but retaining the characteristic(s) of interest for future use." As used in this guidance, the term "kill curve" means "the results of a dose-response experiment where a select agent, toxin or regulated nucleic acids are subjected to increasing amounts of the inactivating treatment to determine the minimum conditions required to render it non-viable or nonfunctional."

Inactivation

In general, the inactivation methods utilized to render a select agent or regulated nucleic acids non-viable or a select toxin nonfunctional fall into one of two broad categories: physical (e.g. heat or radiation) or chemical[3]. Different processes (e.g. heat, radiation or chemicals) work by different mechanisms. Thus for a select agent, regulated nucleic acids, or toxin to be properly characterized as non-viable or nonfunctional after treatment, exposure to the agent, nucleic acids or toxin must not result in infection or toxicity.

A select agent, regulated nucleic acids or toxin[1] must not be treated as non-viable or nonfunctional until it has been subjected to an inactivation method **that has been validated** to be effective on the specific agent, nucleic acids or toxin. **The responsibility of validating non-viability and nonfunctionality remains on the individual or entity possessing the select agent, regulated nucleic acids, or toxin.**

An entity must use a validated inactivation method to render a select agent or regulated nucleic acids non-viable or a select toxin nonfunctional. This means that the inactivation method must be scientifically sound and that it will produce consistent results each time the inactivation method is used such that the expected result can be ensured. An entity's validation of an inactivation method that renders a select agent or regulated nucleic acids non-viable or a select toxin nonfunctional may include 1) use of the exact conditions of a commonly accepted method that has been validated in house as applied, such as autoclaving, 2) a published method with adherence to the exact published conditions that has been validated in-house as applied, or 3) for in-house derived methods, validation testing should include the specific conditions that will be used to prepare subsequent samples (including appropriate positive and negative controls) to verify that the inactivation procedure would be an effective method each time it is performed.

An entity should validate in house their inactivation method with kill curves that include the specific conditions that will be used to prepare subsequent samples. Examples of conditions that should be considered are listed below. Please note this is not an all-inclusive list:

- Exposure or incubation time with inactivant

[3] Biological Safety: Principles and Practices, Diane O. Fleming and Debra L. Hunt, ASM Press.

DRAFT

- Concentration of inactivant
- Concentration of starting material containing select agent, regulated nucleic acids or toxin
- Mixing method and time
- Incubation temperature
- Volume of material

If there is potential for strain-to-strain variations in resistance of a select agent to the inactivation method, then a specific kill curve should be developed for each strain that undergoes the inactivation method.

If there is any deviation or modification to a previously validated inactivation method, including equipment, a revalidation of the method (including kill curve, and viability test) should be done using the new conditions to ensure the method still achieves the appropriate result. In addition, ongoing oversight of inactivation methods should occur to ensure adherence to validated inactivated methods.

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Viability Testing

In addition, a validated viability testing method to ensure that the inactivation method has rendered a select agent or regulated nucleic acids non-viable or a select toxin nonfunctional should be conducted. Validated viability testing methods to verify an agent (including regulated nucleic acids) as non-viable or toxin nonfunctional may include, but are not limited to, cell viability assays, growth analysis, and/or *in vivo* exposure. If possible, for initial validation of the inactivation method, 100% of the sample should be tested to ensure that the sample is completely non-viable or non-functional. Any failures in viability testing or inconclusive results should generate an assessment of procedures followed and possibly a revalidation of the inactivation method if no departure from the prescribed methods is identified. Failure of viability testing when methods are accurately followed is an indication that the method is not sufficiently reliable to be used for inactivation.

It is recommended that an entity maintain information on file in support of the inactivation method used for rendering a select agent or regulated nucleic acids non-viable or a select toxin nonfunctional so that the entity is able to demonstrate that the agent, nucleic acids or toxin are no longer subject to the SAR.

Neutralization

Chemical treatments or antimicrobial activity in inactivated samples may interfere with the viability test. Therefore, viability tests should be performed once chemical or antimicrobial treatments have been subjected to a neutralizing substance or have been shown not to interfere with the viability test. To determine if the chemical treatment or antimicrobial activity is affecting the viability test the following positive control should be performed: Split the sample into two portions, and to one, add live organism to determine if the chemical or antimicrobial activity interferes with the viability test.

Scenarios

Example 1

Question: A laboratory uses formaldehyde to inactivate *Francisella tularensis*, and wants to ship these inactivated cells to unregistered entities. They plan on changing the concentration of cells to be inactivated. Should the entity revalidate their inactivation procedure?

Answer: Yes, any time there has been a change (e.g. equipment changes, different strains used, a change in the volume or concentration of cells) to an inactivation procedure the protocol needs to be revalidated to ensure there are no viable cells present in the sample. A kill curve should be generated to determine the conditions needed to inactivate the material. If there are strain-to-strain variations in sensitivity to the inactivation procedure, kill curves should be generated for all strains. Also, a validated viability testing procedure should be used to verify the effectiveness of the inactivation procedure. In addition, a step to neutralize formaldehyde and positive controls should be included to ensure that the chemical inactivant (formaldehyde) does not interfere with the viability test.

Example 2

Question:

A laboratory in the U.S. has received highly pathogenic avian influenza virus (HPAI) samples from Vietnam under a valid APHIS/CDC Form 2 Transfer authorization. What can the laboratory do in order to safely perform a real-time reverse transcriptase PCR assay on these samples in its unregistered BSL-2 laboratory to further analyze the avian influenza A viral RNA?

Answer:

HPAI is an agricultural select agent. Thus, the initial work with the samples would be conducted in a BSL-3 laboratory with enhancements. Since nucleic acids that encode for HPAI are not subject to the select agent regulations, the use of a validated protocol that extracts nucleic acids and renders this virus nonviable would permit the subsequent work to be conducted in an unregistered BSL-2 laboratory.

Example 3

Question:

A registered entity is inactivating 500 milliliters (mls) of *Yersinia pestis* by gamma irradiation and performs viability tests that show no growth of *Yersinia pestis*. During initial validation of this inactivation method, the entity validated viability on 150 mls of *Yersinia pestis*. Can the 500 ml preparation be worked with as non-select agent material outside of registered space?

Answer:

DRAFT

No, the entity tested viability on material that did not represent the exact conditions (volume) set by the initial validation of inactivation. The entity would have to generate a kill curve to demonstrate that the protocol will inactivate a 500 ml sample.

Example 4

Question:

A registered ABSL-3 laboratory extracts nucleic acid from cell cultures infected with Rift Valley fever virus (RVFV) using a commercially available kit. The entity would like to distribute the nucleic acids to several US laboratories to assist them in the development of *in-vitro* assays. The laboratory would like to know what it should consider when validating that the kit inactivates the RVFV present in the sample.

Answer:

The entity should perform viability tests to validate that the kit inactivates this virus and no select agent material remains. When validating the ability of the kit to inactivate RVFV in-house, the entity should consider viability test conditions such as the use of permissive cells to culture material, appropriate controls, and incubation time of the culture. Once the entity has validated that the kit inactivates RVFV, the nucleic acids, which are not subject to the select agent regulations can be distributed to unregistered laboratories.

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