Inactivation Validation

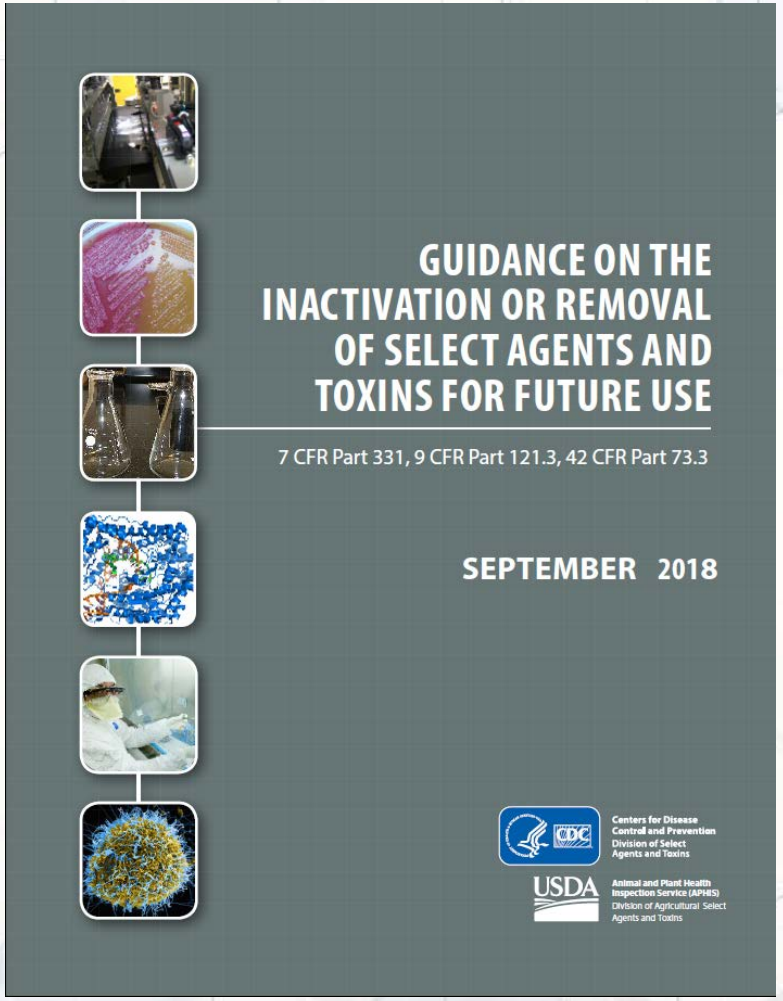
Responsible Official Webinar Series

September 15, 2021

Inactivation of Select Agents

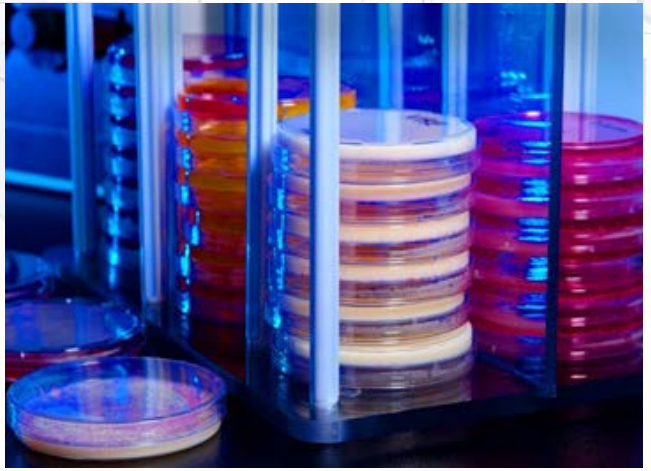
- Entities must confirm their select agent inactivation or select agent removal procedures in-house via viability testing.

- Guidance on how to develop and validate procedures and protocols and verify inactivation or select agent removal can be found at https://www.selectagents.gov/irg-intro.html.



Inactivation Regulatory Definitions

Validated inactivation procedure is a procedure, conducted in-house whose efficacy is confirmed by data generated from a viability testing protocol, to render a select agent non-viable but allows the select agent to retain characteristics of interest for future use, or to render any nucleic acids that can produce infectious forms of any select virus non-infectious for future use.



Inactivation Regulatory Definitions

Viability testing protocol means a protocol to confirm the [in-house] validated inactivation procedure by demonstrating the material is free of all viable select agent.

Inactivation Regulations

Section 3 (d) Select agents that meet any of the following criteria are excluded from the requirements of this part:

- Section 3 (d)(4): A select agent or regulated nucleic acids that can produce infectious forms of any select agent virus that has been subjected to a validated inactivation procedure that is confirmed through a viability testing protocol.

- Section 3 (d)(5): Material containing a select agent that is subjected to a procedure that removes all viable select agent cells, spores, or virus particles and the material is subjected to a viability testing protocol to ensure that the removal method has rendered the material free of all viable select agent.

Select Agent Inactivation In-House Validation

In-house validation of an inactivation procedure can occur with:

- An entity-derived procedure with specific conditions.
- A published procedure with adherence to the exact published conditions.
- The exact conditions of a commonly accepted procedure.

Procedure Validation

- Procedure validation can be accomplished by viability or infectivity testing.
- An entity must:
  - Include the use of appropriate positive, negative, and process controls.
  - Perform sufficient experimental replicates to determine inherent variability with the procedure.
- The number of replicates will vary depending on the procedure and agent, and it is the entity's responsibility to determine a sufficient number of replicates.

Procedure Validation

Examples of items to **consider** when validating an inactivation or select agent removal procedure are listed below:

- Adherence to standardized extraction kit instructions for nucleic acids, proteins, polysaccharides, etc.
- Appropriate assay for the starting material (virus, vegetative bacteria, or spores).
- Limit of Detection/Limit of Quantification of the viability testing protocol.
- Concentration of starting material containing select agent and regulated nucleic acids (start with highest concentration expected as a worst-case scenario and then set that concentration as the upper limit for subsequent inactivation). Also, using a higher concentration than expected allows for a safety margin to be incorporated.
- Matrix materials that could interfere with viability tests.
- Validation with all (100%) of the sample (for large volume cultures you can filter 100% of the sample and then culture the filter).
- Need for neutralization of chemical or antimicrobial treatments.

Select Agent Inactivation In-House Validation

- Verify validated inactivation procedure based on entity risk assessment.
- When validating an inactivation procedure, inclusion of safety margin is recommended to ensure complete inactivation.
- Perform risk assessment to determine a sampling strategy for verification viability or infectivity testing for subsequent inactivation.
  - Verification viability testing means testing conducted on samples that have been subjected to a validated inactivation or removal procedure, to confirm the material is free of viable select agent, or nucleic acids of any select agent virus capable of producing infectious virus.

Use of Surrogates for Inactivation Validation

- The select agent regulations provide that surrogate strains that are known to possess equivalent properties with respect to inactivation can be used to validate an inactivation procedure.
  - Viruses from the same **family** can be suitable surrogates for select agent viruses,
  - Bacteria from the same **genus** can be suitable surrogates for select agent bacteria, and
  - Any positive single stranded RNA can be suitable surrogates for regulated positive single stranded RNA.
- If there are known strain-to-strain variations in the resistance of a select agent to an inactivation procedure, then an inactivation procedure validated on a lesser resistant strain must also be validated on the more resistant strains.

Bacillus anthracis and *Bacillus cereus* biovar *anthracis* Inactivation Policy

Inactivated Sample Created Using One of the Following Methods

Chemical: Cells or spores

Must test 100% of sample during initial validation.

Determine residual chemical effect on viability testing and incorporate neutralization if necessary.

Test ≥10% of the sample or lot. Place in broth for at least 7 days.

Test ≥100 µl of broth onto an agar plate for at least 7 days.

Verification Viability Testing

Chemical: Tissues

Must test 100% of sample during initial validation.

Determine residual chemical effect on viability testing and incorporate neutralization if necessary.

Viability testing NOT required for every sample if using a validated inactivation procedure.

Heat

Must test 100% of sample during initial validation.

Use validated autoclave temperature and time that includes safety margin

For subsequent samples, use a *Bacillus* spore-based indicator when inactivating samples.

Filtered Extracts

Must test 100% of sample during initial validation.

Must use 0.22 µm or smaller filter.

Test ≥10% of the sample or lot. Place in broth for at least 48 hrs.

Test ≥100 µl of broth onto an agar plate for at least 48 hrs.

Additional Requirements in Inactivated *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* Policy

	All agents and regulated nucleic acid except *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis*	***Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis***
Initial validation (includes viability test)	To be determined by entity (volume of test material, broth/agar, culture duration, temp, etc.)	100% of the inactivated material, or filter (pore size ≤ 0.22 micron) 100% of the inactivated material, then culture the filter then follow viability testing as described in verification column
Safety margin	Recommended	Required

Additional Requirements in Inactivated *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* Policy

	All agents and regulated nucleic acid except *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis*	***Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis***
Verification viability testing	It depends. Sampling strategy based on entity risk assessment except for samples where agent is <u>only</u> removed. That material requires verification viability testing on every sample.	≥10% of inactivated material directly inoculated into a broth medium. For large volume cultures, use a 0.22 µm filter to filter ≥10% of the inactivated material and culture the filter. Incubate for ≥ 48 hours (7 days for chemical inactivation) at 35º±2ºC, and then plate ≥ 100 microliters of broth culture onto agar plate, incubate at 35°±2ºC ≥ 48 hours (7 days for chemical inactivation). For autoclaved samples use an appropriate *Bacillus* species spore-based indicator.

Additional Requirements in Inactivated *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* Policy

	All agents and regulated nucleic acid except *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis*	***Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis***
Determination if residual inactivating chemical interferes with viability test (is removal or neutralization required?)	Recommended	Required Split the chemically treated sample into two portions. To one, add ≥100 *B. anthracis* (e.g. Sterne, Pasteur, Ames) spores. If the residual chemical or antimicrobial activity interferes with the viability test, then use neutralization methods initially validated by using 100% of the sample.

Inactivated *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* Policy

	All agents and regulated nucleic acid except *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis*	***Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis***
Investigation of inactivation or viable select agent removal failures	Required	Required
Annual review		
Records		
Waiver		

Inactivation of Regulated Genomic Material

Validation of an inactivation procedure for a positive-strand RNA virus (e.g., SARS-CoV, Eastern Equine Encephalitis virus), but not including inactivation of the regulated genomic material:

- Genomic material capable of forming a regulated infectious virus is also regulated.
- For a sample to be excluded from the select agent regulations, the virus and the genomic material must be rendered non-infectious using a validated inactivation procedure.
- Initial in-house validation of the inactivation procedure must be performed for both the virus and the regulated genomic material.

Chemical Inactivation of Select Agents

- Presence of residual chemicals in samples could skew the results of the viability test causing false negatives.
- Determination if removal/neutralization of inactivating chemical (e.g., TRIzol, formalin, lysis buffers, antimicrobial agents) is required prior to viability testing:
  - Required for *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis*
  - Highly recommended for all other agents
- Suggested methods for removal of chemical effects:
  - Washing of cells with buffer/water
  - Neutralization
  - Spin columns
  - Dialysis
  - Dilution

When Should You Revalidate Your Procedure?

Examples of when revalidation would be required

- If the procedure is significantly changed. For example:
  - New type of extraction kit with different buffers
  - Change in reagent
  - New type of equipment used to inactivate select agent
- If there is a change in the procedure due to a failure
- If a higher concentration/titer is inactivated than was validated

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