


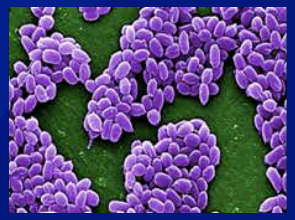

Inactivation of Select Agents

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USDA

CDC

The Inactivation Regulatory Provisions Do Not Apply To:

- Non-viable select agents or non-toxic toxins.
- Select agents excluded as an attenuated strain of a select agent, a toxin modified to be less potent or toxic.
  - A list of excluded agents can be found at https://www.selectagents.gov/SelectAgentsandToxinsExclusions.html.
- Select agents and toxins subjected to a decontamination or destruction procedure for waste disposal.
- Select toxins.

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 whose efficacy is confirmed [in-house] 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 if 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 inactivation procedure validation by an entity may include the validation of the following:
  - Use of entity-derived procedure with specific conditions.
  - Use of a published procedure with adherence to the exact published conditions.
  - Use of the exact conditions of a commonly accepted procedure.

Select Agent Inactivation In-House Validation

- Entity must confirm their inactivation or select agent removal procedure in-house:
  - Use appropriate positive, negative, and process controls to determine if the procedure works as intended.
  - Use the final inactivation conditions derived from the procedure development step or existing procedure and viability test for the absence of viable organism.
  - Perform sufficient experimental replicates to determine inherent variability with the procedure.

Select Agent Inactivation In-House Validation

- Verify validated inactivation procedure based on entity risk assessment.
- When validating inactivation procedure, inclusion of safety margin is recommended to ensure complete inactivation.
- Perform risk assessment to determine a sampling strategy for viability or infectivity testing for subsequent inactivation.

Select Agent Verification Viability Testing

- The requirement for verification viability testing of subsequent samples will differ depending on the category of the sample.

Sample category	Verification required after sample subjected to validated inactivation or select agent removal procedure
Inactivated Agent (Cell cultures, tissue samples, etc.)	It depends, sampling strategy developed by entity based on risk assessment.
Extracts (nucleic acids, proteins, polysaccharides, etc.)	
Material containing select agents that is subjected to a process to remove (e.g. filtration) all viable cells, spores, or virus particles	Yes, on all samples

Annual Review of Inactivation Procedures

- **The Responsible Official (RO) must [Section 9 (a)(9)]:**
  - **Review, and revise as necessary, each of the entity's validated inactivation procedures or viable agent removal methods.**
- **The review must be conducted annually or after any:**
  - Change in principal investigator (PI).
  - Change in the validated inactivation procedure or viable agent removal method.
  - Failure of the validated inactivation procedure or viable agent removal method.
- **The review must be documented and training must be conducted if there are any changes to the validated inactivation procedure, viable agent removal method, or viability testing protocol.**
- **The annual review requirement does not necessarily involve revalidating inactivation procedures.**

Reporting Requirements for Inactivation Failures

- The RO must investigate to determine the reason for any failure of a validated inactivation procedure or any failure to remove viable agent from material [Section 9(a)(8)].

- The RO must report immediately by telephone or email failure of the validated inactivation procedure or viable agent removal to FSAP if:

  - The RO cannot determine the cause of a failure of a validated inactivation procedure or a viable agent removal method, or
  - A report is received of an inactivation failure after the movement of material to another location.

Record Requirements for Inactivation Procedures
Section 17(a)(8)

- A written description of the validated inactivation procedure or viable select agent removal method used, including [in-house] validation data.
- A written description of the viability testing protocol used.
- A written description of the investigation conducted by the entity RO involving a procedure failure and the corrective actions taken.

Record Requirements for Inactivation Procedures Section 17(a)(8)

- The name of each individual performing the procedure.
- The date(s) the procedure was completed.
- The location where the procedure was performed.
- A certificate, signed by the PI, that includes the:
  - Date of inactivation or viable select agent removal
  - Validated inactivation or viable select agent removal method used
  - Name of the PI

Applying For A Waiver

- A select agent or regulated nucleic acid that can produce infectious forms of any select agent virus not subjected to a validated inactivation procedure, or material containing a select agent not subjected to a procedure that removes all viable select agent cells, spores, or virus particles, may be excluded if the material is determined by the HHS Secretary or APHIS Administrator to be effectively inactivated or effectively removed.

- To apply for a determination, an individual or entity must submit to FSAP:
  - A justification regarding the alternative procedure including a description of what material is to be waived.
  - The inactivation/removal procedure and viability test to be used.
  - Validation data.
  - Any other supporting information, such as scientific references.

- A written decision granting or denying the request will be issued.

- Additional information on applying for a waiver can be found at: https://www.selectagents.gov/irg-intro.html.

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

https://www.selectagents.gov/regulations/policy/bacillus.htm

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.

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 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

Agent	All agents and regulated nucleic acids	*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

Agent	All agents and regulated nucleic acids	*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 only 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

Agent	All agents and regulated nucleic acids	*Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis*
Neutralization	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.

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

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

POLICIES

Deviation From a Validated Inactivation Procedure or Removal Method

(May 12, 2017)

https://www.selectagents.gov/policystatement_deviation.html

- Section 9(a)(8): Investigate to determine the reason for any failure of a validated inactivation procedure or any failure to remove viable select agent from material. If the Responsible Official is unable to determine the cause of a deviation from a validated inactivation procedure or a viable select agent removal method; or receives a report of any inactivation failure after the movement of material to another location, the Responsible Official must report immediately by telephone or email the inactivation or viable agent removal method failure to CDC or APHIS.

- Policy: It was and is the intent of the FSAP that the use of the phrase "a deviation from a validated inactivation procedure or a viable select agent removal method" means "a failure of a validated inactivation procedure or a viable select agent removal method."

Chemical Inactivation of Whole Tissue or Homogenized Tissue (February 13, 2018)

https://www.selectagents.gov/policystatement_tissueinactivation.html

- In meeting the in-house validation/verification of inactivation by chemical inactivation of whole tissue or homogenized tissue, it is FSAP policy to allow entities to select one tissue type either:
  - The tissue that is expected to have the highest concentration of the specific agent to be inactivated

    OR

  - Determine agent concentration for the agent to be inactivated in a tissue before performing inactivation to use as the maximum limit for that agent to serve as a surrogate for other tissues, including those in other animal models, so long as all standardized conditions are held constant such as the agent used, tissue size, and ratio of tissue to volume of inactivating chemical.

Chemical Inactivation of Whole Tissue or Homogenized Tissue (February 13, 2018)

https://www.selectagents.gov/policystatement_tissueinactivation.html

- A safety margin must be incorporated into the final chemical inactivation procedure to ensure the effective inactivation of the agent.

Certificate Meaning and PI Signature (August 3, 2018)

https://www.selectagents.gov/policystatement_certificate.html

- The purpose of the certificate is for the PI, or designee, to certify that the information contained on the certificate is correct.
- The signature of the PI, or designee, on the certificate will certify that the information listed is true, complete, and accurate.
- The certificate must be signed by the appropriate PI, or designee, before the "inactivated material" is removed from registered space or the biocontainment level required for that material.

Select Agent Inactivation In-House Validation (August 10, 2018)

https://www.selectagents.gov/policystatement_inactivation.html

- In-house validation must be completed prior to the use of the procedure to render a select agent non-viable for future use or to render any nucleic acids that can produce infectious forms of any select agent virus non-infectious for future use.

Select Agent Inactivation In-House Validation (August 10, 2018)

https://www.selectagents.gov/policystatement_inactivation.html

- **An entity can:**
  - Use exact conditions of a commonly accepted procedure (such as autoclaving) whose efficacy is confirmed with data generated from a viability testing protocol (validated) in-house by the entity.
  - Use a published procedure with adherence to the exact published conditions whose efficacy is confirmed with data generated from a viability testing protocol (validated) in-house by the entity.
  - Use an entity-derived procedure with specific conditions whose efficacy is confirmed with data generated from a viability testing protocol (validated) in-house by the entity.

Exclusion of Formalin-Fixed, Paraffin-Embedded Tissues (October 9, 2018)

https://www.selectagents.gov/policystatement_formalintissue.html

- A select agent or regulated nucleic acids that can produce infectious forms of any select agent virus contained in a formalin-fixed, paraffin-embedded (FFPE) tissue has been effectively inactivated if the FFPE process used is a recognized method (e.g., a previously published method shown to be effective such that validation does not have to occur in house for FFPE tissues) for that particular agent or regulated nucleic acids.

- It is therefore the policy of the FSAP that such material is not subject to the select agent regulations.

REGULATORY INTERPRETATIONS

Surrogates (April 21, 2017)

https://www.selectagents.gov/reg-int_surrogate-strains.html

- 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.

PI Signature on Certificate

(April 11, 2017)

https://www.selectagents.gov/policystatement_pi.html

- The PI is the one individual who is designated by the entity to direct a project or program and who is responsible to the entity for the scientific and technical direction of that project or program (including all inactivation procedures or removal procedures associate with that project).

- In the absence of that PI, the individual designated by that PI and approved by the entity RO to sign the certificate in his or her absence.

- In order for an individual to be the PI's designee to sign the certificate, a person must be listed on the entity's registration and have the knowledge and expertise to provide scientific and technical direction regarding the validated inactivation procedure or the procedure for removal of viable select agent to which the certificate refers.

Certificates (May 17, 2019)

https://www.selectagents.gov/reg-int_certificates.html

Does a copy of the PI-signed inactivation certificate need to accompany any transfer outside of containment?

- A copy of an inactivation certificate must accompany the inactivated material when the inactivated material is transferred externally (from your entity to another entity).
- It is recommended that an inactivation certificate also accompany the transfer of inactivated material internally (from one PI to another PI at the same registered entity).
- Additionally, regardless of whether a transfer is made, an entity remains responsible for the record keeping requirements found in Section 17(a)(8) of the select agents and toxins regulations.
- An original certificate must be generated for every sample inactivated regardless of future transfer.
- FSAP recommends that entities maintain the certification of inactivation as long as the material is in their possession.

Certificates

Does a certificate have to be generated if the inactivated select agent or regulated nucleic acids or select agent removed material stays within registered space?

- Yes. For each select agent that has been subjected to a validated inactivation procedure or a procedure for removal of viable select agent, a registered entity must generate a certificate, signed by the PI, that includes the date of inactivation or viable select agent removal, the validated inactivation or viable select agent removal method used, and the name of the PI.
- The location of the inactivated or select agent removed material does not change the requirement to generate a certificate.

Other Relevant Decisions

Electronic Signature-Certificates

(SA Gram 9/7/18)

- For electronic signature, the method used should:
  - Identify and authenticate a person using at least two factors of authentication, including something the person knows (i.e., email password) and something the person has (e.g., a mobile phone with SMS text message access).
  - Provide a means to preserve the integrity of the signed record that is (a) portable, (b) independently verifiable, (c) tamper-evident, (d) granular, and (e) verifiable in the long-term.
- The electronic signature should also:
  - Capture the intent, such as indicate a person's approval of the document.
  - Be backed by an audit trail.

INACTIVATION COMPLIANCE ISSUES

Inactivation Compliance Issues

- Surrogates: Entity validated the inactivation procedure with one family of viruses and then used it to inactivate other viral families.
  - Corrective action: Required entity to follow policy for surrogates. Viruses from the same family can be suitable surrogates for select agent viruses.

- Certificates: PI signing certificates before the inactivation procedure is performed. PI has approved of the experiment and outside of initial validation, there is no requirement for verification viability testing, therefore the entity determined that the certificate can be signed before inactivation is performed.
  - Corrective action: Required entity to sign certificates after inactivation has occurred.

Inactivation Compliance Issues

Inactivation validation:

- Volumes of inactivated material tested during viability testing were insufficient, but FSAP does not have a requirement for volumes that must be tested.
  - Corrective action: Requested the entity justify the volumes used in their viability testing and indicate how the entity would be able to detect an inactivation failure.

- Verification viability testing only without initial validation
  - Corrective action: Resulted in DSAT issuing a policy (8/10/18) clarifying that in-house validation must be completed prior to the use of the procedure.

Inactivation Compliance Issues

Inactivation validation:

- Use of published formalin penetration rates in an inactivation procedure without validating the rates/procedure in house.
  - Corrective action: Required entity to validate rates/procedure in house.

- Use of published gamma irradiation inactivation curve as basis for the irradiation times in an inactivation procedure without validating the procedure in house.
  - Corrective action: Required entity to validate rates/procedure up front in house.

- Use of an inactivation procedure on a higher titer virus than what was used when validating the procedure.
  - Corrective action: Required entity to validate the inactivation procedure in house with the higher titer or only use the validated procedure on titers equal to or less than what was used during validation.

Inactivation Compliance Issues

- Validation of an inactivation procedure for a positive-strand RNA virus (e.g., SARS-CoV, EEE, VEE), but not including inactivation of the regulated genomic material:
  - Genomic material from these viruses capable of forming an infectious virus is 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

- Removal/neutralization of inactivating chemical (e.g., TRIzol, formalin, lysis buffers, antimicrobial agents) prior to viability testing
  - Required for *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis*
  - Highly recommended for all other agents

- Presence of residual chemicals in samples could skew the results of the viability test causing false negatives

- Suggested methods for removal of chemical effects:
  - Washing of cells with buffer/water
  - Neutralization
  - Spin columns
  - Dialysis
  - Dilution

Discussion

www.selectagents.gov

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APHIS: AgSAS@usda.gov or
301-851-3300 option 3 (voice only)