


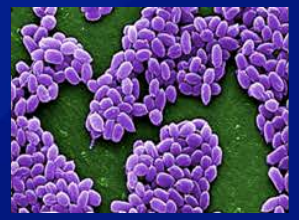

Federal Select Agent Program (FSAP)

Responsible Official Workshop
August 15-17, 2018

INACTIVATION OF SELECT AGENTS AND REGULATED NUCLEIC ACIDS

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.

THE INACTIVATION REGULATORY PROVISIONS DO NOT APPLY TO:

- Non-viable select agents or non-toxic toxins.
- Select agents and toxins 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 REGULATIONS

- (d) Select agents that meet any of the following criteria are excluded from the requirements of this part (42 CFR Part 73):
  - 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.
    - *Surrogates*
  - 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.

SURROGATES

- Ideally, an inactivation procedure should be developed with the exact organism it is intended for. However, there may be occasions when this is not possible.
- If there are known strain-to-strain variations in resistance to an inactivation procedure, then an inactivation procedure must be developed using the more resistant strain.
- It is up to the entity to determine any strain resistance to a particular inactivation method and use appropriate surrogates.
  - Suitable surrogates:
    - Viruses from the same family
    - Bacteria from the same genus
    - Any positive single strand RNA genome can be used to represent regulated positive single strand RNA genomes

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.

- 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.

SELECT AGENT INACTIVATION IN-HOUSE VALIDATION

- Inactivation procedures used to inactivate a select agent or nucleic acid that can produce infectious forms of any select agent virus must be validated in-house prior to their use.

- Efficacy of inactivation is confirmed by data generated from a viability testing protocol.

SELECT AGENT INACTIVATION IN-HOUSE VALIDATION

1. In-house validation by an entity of an inactivation procedure may include:
    1. Use of entity-derived procedure with specific conditions.
    2. Use of a published procedure with adherence to the exact published conditions.
    3. Use of the exact conditions of a commonly accepted procedure (such as autoclaving).
2. 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.

INACTIVATION PROCEDURES (CONT.)

3. Verify validated inactivation procedure based on entity risk assessment.
4. Perform risk assessment to determine a sampling strategy for viability or infectivity testing for subsequent inactivation.
5. Verification of inactivation or removal procedures will differ depending on the category of the sample: 1) inactivated agent, 2) extracts, or 3) material.

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:
  - 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.

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

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

RECORD REQUIREMENTS FOR INACTIVATION PROCEDURES

- 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.
- 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
- A copy of the certificate must accompany any external transfer (entity to entity) of inactivated or select agent removed material.

CERTIFICATES

- Typically, the PI signs the inactivation certificate, which must contain the date of inactivation or removal, method used to inactivate or remove, and the PI's name. The signature certifies that the information regarding the inactivation of the material has been reviewed and that the results are as intended.

- In the absence of a PI, an individual designated by the PI and approved by the entity's RO can sign the certificate on his or her behalf.

- In order for an individual to be the PI's designee to sign the certificate, the person must:
  - Be listed on the entity's registration.
  - 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

- The signature of the Principal Investigator, or designee, on such certificate will certify that the information listed on that certificate is true, complete, and accurate.
- No specific prescribed format is required; the only requirement is that the documentation contains the information required by the regulations.
- Information can be added to existing documentation.
- This documentation can be batched and recorded outside of containment.
- The documentation must be signed after inactivation has occurred.
- A copy of the certificate is required only for transfers between entities.

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.

INSPECTION

- For material where the select agent was removed (i.e., filtration) without a prior inactivation step, a portion of every sample must be verified by viability testing. The portion (recommend between 5-10%) of the sample used in viability testing is up to the entity based on their risk assessment.

- For all other types of samples (e.g., fixed tissues, extracts, lysates, chemically treated cultures), verification viability testing is recommended but not required by the regulations.
  - For any type of inactivated samples where entities do not have verification viability test results, inspectors will ask for and review the viability test results from the experiments used to initially validate the inactivation procedure to determine if the conditions selected would completely inactivate the material (i.e., no growth).

INSPECTION

- Inspectors will ask for and review viability test results (validation or verification).
  - For entities that perform inactivation of samples, inspectors may review results of a portion of the inactivated material in a fashion similar to BSAT inventory audits.
  - For those viability test results that show positive growth (i.e., an inactivation failure), but the entity has determined and documented the reason for the inactivation failure (and has made the corrective actions needed), and has not removed inactivation failure material outside of registered space, then no further action is taken by inspectors. Inspectors will not collect validation data in these situations.

INSPECTION

- Inspectors will ask for and review viability test results (validation or verification).
  - If a cause for the inactivation failure is not identified by the entity, inspectors will collect the protocols and validation data (to include data on kill curves or bioburden reduction experiments, or other experiments used to validate an inactivation procedure) to determine the adequacy of the inactivation procedure. Inspectors will verify the material that failed inactivation remained in registered space and was not manipulated outside of primary containment.
- It is up to the entity to determine any strain specific impact (resistance) on inactivation method and use appropriate surrogates.
  - Inspectors will ask if there are known strain-to-strain variations in the resistance of a select agent to an inactivation procedure.

POLICIES AND REGULATORY INTERPRETATIONS

- Application of the requirement for a “validated inactivation procedure” as used in the select agent regulations
- Inactivation Certificate
- Chemical inactivation of whole tissue or homogenized tissue
- Application of the requirement for a “validated inactivation procedure”
- Meaning of the phrase “a deviation from a validated inactivation procedure or a viable select agent removal method”
- Requirement for inactivation certificates and intra-entity transfers
- Signature by “principal investigator” on inactivation certificates
- Surrogate strains which can be used to validate inactivation procedures

INACTIVATION OF *BACILLUS ANTHRACIS* AND *BACILLUS CEREUS* BIOVAR *ANTHRACIS*

Exclusion categories:

- Preparations of regulated strains of *B. anthracis*, including *B. anthracis* Pasteur, that were subjected to an inactivation procedure prior to June 2, 2015
- Upon meeting specific conditions described in the Inactivated *Bacillus anthracis* and *Bacillus ceres* Biovar *anthracis* policy:
  - Chemically-treated vegetative cells and spore preparations
  - Chemically-treated whole tissue specimens (such as formalin-fixed tissue)
  - Heat treated (autoclave) vegetative cell and spore preparations
  - Extracts (e.g., nucleic acid extracts, antigens, lysates) from regulated strains of *B. anthracis* or *B. cereus* Biovar *anthracis* or material containing regulated strains of *B. anthracis* or *B. cereus* Biovar *anthracis* (e.g., serum, culture) where viable agent is removed

Comparison of inactivation regulations and inactivated BA & BcBva policy

	Inactivation regulations	Policy August 14, 2017
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

Comparison of inactivation regulations and inactivated BA & Bcbva policy

	Inactivation regulations	Policy August 14, 2017
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.

Comparison of inactivation regulations and inactivated BA & Bcbva policy

	Inactivation regulations	Policy August 14, 2017
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.

Comparison of inactivation regulations and inactivated BA & Bcbva policy

	Inactivation regulations	Policy August 14, 2017
Investigation of inactivation or viable select agent removal failures	Required	Required
Annual review		
Records		
Waiver		

MORE INFORMATION

- Frequently asked questions regarding the inactivation provisions are available at: https://www.selectagents.gov/faq-inactivation.html
- Guidance document regarding inactivation is available at: https://www.selectagents.gov/irg-intro.html
- FSAP Policy Statement on Inactivated *Bacillus anthracis* and *Bacillus cereus* Biovar *anthracis* is available at: https://www.selectagents.gov/policystatement_bacillus.html
- Questions? LRSAT@cdc.gov or AgSAS@aphis.usda.gov

FAQs/Scenarios

FREQUENTLY ASKED QUESTIONS

- Does a select agent or regulated nucleic acid that was subjected to a validated inactivation procedure prior to March 21, 2017 need to be re-validated as inactivated by the entity?
  - *No. The provisions are to be implemented on any samples inactivated on or after the effective date of the regulations and do not apply retroactively. However, entities should follow prudent practices based on risk assessments.*
- Is an annual review required by the RO for the inactivation protocols?
  - *Yes. The review must be conducted annually or after any change in PI for those protocols used by that PI, after any change in a validated inactivation procedure or a viable select agent removal method, or after any failure of a validated inactivation procedure or viable select agent removal method. However, the annual review does not mean the procedures have to be revalidated. For example, the RO could review the inactivation procedures and determine that they are still being used as designed and work as intended. In this situation, revalidation would not be necessary.*

FREQUENTLY ASKED QUESTIONS

- Do I have to develop (and then validate in-house) my own inactivation procedure to meet the requirements, or can I validate an existing procedure in-house to meet the regulatory requirements?

  - *An entity can certainly develop and validate their own inactivation procedure, or the entity can use an already developed (commonly accepted or published) inactivation procedure that the entity validates in-house.*
  - *To validate an inactivation procedure means an entity has performed the inactivation and conducted viability testing of the end product in-house to confirm the efficacy of the inactivation procedure.*

- How many replicates do I need to validate my inactivation procedure?

  - *Entities should use multiple replicates when validating their inactivation procedures. The number of replicates may vary depending on the procedure and agent. It is the entity's responsibility to determine a sufficient number of replicates.*

FREQUENTLY ASKED QUESTIONS

- Do I need to revalidate the inactivation protocol if I plan to use a lower concentration of select agent material being inactivated?
  - *No. However, revalidation would be required if there is a higher concentration of material being inactivated.*
- Who determines the validity of an inactivation protocol?
  - *The responsibility for this activity remains with the entity.*
  - *FSAP inspectors will verify that the entity has validated an inactivation procedure in-house and focus their review on viability test results. However, there may be times (e.g. inactivation failures) when inspectors will request validation data and procedures for review.*

FREQUENTLY ASKED QUESTIONS

- Are nonregistered clinical or diagnostic laboratories and other entities that possess, use, or transfer a select agent that is contained in a specimen presented for diagnosis or verification exempt from the inactivation requirements?

  - *Yes, so long as the select agent meets the following conditions:*
    - *1) is transferred or destroyed (by a recognized sterilization or inactivation process) within seven calendar days after identification.*
    - *(2) is secured against theft, loss, or release.*
    - *(3) is reported to CDC or APHIS, the specimen provider, and to other appropriate authorities when required by federal, state, or local law by telephone, facsimile, or email. This report must be followed by submission of APHIS/CDC Form 4 to APHIS or CDC within seven calendar days after identification.*

  It is strongly recommended that clinical or diagnostic laboratories validate their inactivation procedures in-house to reduce the risk of failure of an inactivation procedure. An inactivation procedure failure resulting in access to a select agent by unapproved individual(s) would be a violation of the select agent regulations and could result in civil or criminal penalties.

FREQUENTLY ASKED QUESTIONS

- If an inactivation procedure is changed, who needs to receive training on the updated procedure?
  - *If changes to the inactivation procedure are made (e.g., due to a failure of the procedure or during review of the procedure), then training must be provided to the individuals that would perform the inactivation procedure. This may be all individuals that act as laboratorians under a PI, or any individuals at the entity that would work with the same agent using the same inactivation procedure.*

INACTIVATION QUESTIONS

- What actions should the RO/ARO take when inactivated QC organisms are: a) incorrectly labeled, b) have no certificate of inactivation, or c) inactivation lot # doesn't match the lot # of the organism received?
  - *Any time the entity inactivates a select agent, an inactivation certificate must be generated and sent with the inactivated sample if shipped to another entity.*
  - *If there are discrepancies between what is requested vs. what is received, it is the entity's responsibility to reconcile with the sender.*
  - *If the record keeping indicates that a current and accurate inventory is not being maintained, then the entity may be cited.*
- In regards to the question above, should/must the RO/ARO try to solve the issue at the lowest level first or report to CDC DSAT immediately?
  - *The RO/ARO is required to investigate inactivation failures, and if they cannot determine a reason for the failure, then they must notify FSAP.*
  - *If the issue is with record keeping, there are requirements to correct the record keeping issues to remain in compliance with the regulations.*

INACTIVATION SCENARIOS

- An entity infects mice with *Francisella tularensis*, and then collects blood for serum at different time points. The entity wants to filter the serum and further use the filtered serum in their unregistered BSL-2. Do they have to viability test this filtered material before transferring it to their BSL-2 laboratory? Does a certificate have to be generated by the entity, and if so, does of copy of the certificate have to accompany the sample to the BSL-2 laboratory?

INACTIVATION SCENARIOS

- *Yes, the entity must viability test all filtered samples (recommend 5-10% of each sample) that do not first have an inactivation step. This is called a select agent removal method. A certificate must be generated for the filtered sample, but a copy of the certificate does not have to accompany the sample to the unregistered laboratory because it is within the same entity.*

INACTIVATION SCENARIOS

- An entity is registered for *F. tularensis, Y. pestis, B. pseudomallei,* and all three *Brucellae*. The entity does not want to validate their heat inactivation procedure for all the organisms since they are all Gram negative bacteria and should react to the heat in the same way. Can the entity only use the excluded strain *B. pseudomallei* Bp82 to validate their procedure, and apply it to all their registered bacteria?

INACTIVATION SCENARIOS

- *No. The entity can develop an inactivation procedure using B. pseudomallei Bp82, but they must still validate the procedure using at least one representative from each genus. If there are not known strain-to-strain variations then use of a surrogate strain within the same genus is acceptable for bacteria. For instance, the entity can use the excluded strain B. abortus strain RB51 during the initial validation, and apply it to all the Brucellae (B. abortus, B. suis, B. melitensis) if the entity can reasonably assume all strains would react to the inactivation procedure the same.*

INACTIVATION SCENARIOS

- An entity wants to chemically inactivate *Burkholderia pseudomallei* and use the inactivated cells in an unregistered laboratory for vaccine development. During initial validation of the inactivation procedure, does the entity have to viability test 100% of the sample? What percentage does the entity have to test of the subsequent samples using the validated procedure?

INACTIVATION SCENARIOS

- *For all agents except Bacillus anthracis and Bacillus cereus Biovar anthracis, the % of sample used to verify the inactivation is up to the entity (recommend 5-10%), which should be based on a risk assessment. We do encourage entities to test 100% of the sample during initial validation, but it is not required. The % of sample from subsequent inactivation experiments used to verify the removal of viable organisms is up to the entity, which should also be based on a risk assessment.*

INACTIVATION SCENARIOS

- An RO is notified by a laboratorian that a sample that was thought to be inactivated in a registered BSL-3 had been transferred to an unregistered laboratory. The sample was created using a validated inactivation procedure, and the inactivation certificate for the sample was signed by the PI. No viability test was performed on this sample since the entity had determined with a risk assessment that viability testing on every sample was not required. What steps must the RO take once this sample was determined to be viable?

INACTIVATION SCENARIOS

- *The sample must be taken back into the registered space and secured.*
- *The entity must notify FSAP with an APHIS/CDC Form 3 that a viable select agent was taken to unregistered space, and describe any manipulation of the agent that occurred and any potential exposures.*
- *The RO must perform an investigation into why the validated inactivation procedure failed, and document the investigation and any corrective actions taken.*
- *If the reason for the failure is determined, then any changes that are made to the procedure must be revalidated and training on the changes must be provided to the entity staff that would use or perform the inactivation procedure.*
- *If the reason for the failure cannot be determined, then the RO must immediately contact FSAP and provide details on the investigation. FSAP will then work with other SMEs to try and determine why the failure occurred.*

INACTIVATION SCENARIOS

- A PI has validated an inactivation procedure that uses formalin to fix spleens from mice. Spleens were selected for the initial study because the agent concentrates in this tissue regardless of the animal model used. The PI has now decided to take other tissues from infected mice, rats, and guinea pigs and fix them with formalin. Does the PI have to validate the inactivation procedure for each tissue from each type of animal?

INACTIVATION SCENARIOS

- *No. The validated procedure used to formalin fix the spleens can be used for all other tissues from any animal 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. A safety margin must be incorporated into the final chemical inactivation procedure to ensure the effective inactivation of the agent.*

INACTIVATION SCENARIOS

- If a new bioreactor is installed, do new inactivation parameters need to be validated or can inactivation processes with the previous bioreactor be used?

INACTIVATION SCENARIOS

- *New parameters need to be validated and the laboratorian needs to be aware of structural differences in the new bioreactor which may affect inactivation effectiveness. Examples include ports where cells could fall into a “dead zone” and not be thoroughly mixed with the cell suspension during inactivation. Also, be aware of live cells which may be aerosolized into the exhaust filter and may fall with condensation into the heat-inactivated cell suspension.*

INACTIVATION SCENARIOS

- If a large sampling volume (750 – 1000 mL), to be used for inactivation testing, is collected due to serial samplings from a 90L bioreactor bacterial cell production process, how much of the collected sample volume must be inoculated for viability testing?

INACTIVATION SCENARIOS

- *There is no set regulatory volume that should be sampled. However, the volume used for viability testing should ensure representative sampling of the collected volume and the viability test protocol should ensure the limit of detection is optimized along with any media used for the viability testing process. An enrichment media along with possible extended incubation periods of the collected sample volume may assist with an improvement to limits of detection for the viability testing procedure.*
- *Alternatively, filtering of large sample volumes (750-1000ml) and then culturing the filter could be performed to avoid plating large volumes.*

INACTIVATION QUESTIONS?

FEDERAL SELECT AGENT PROGRAM

www.selectagents.gov

CDC: lrsat@cdc.gov or 404-718-2000

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