U.S. Department of Health and Human Services
Centers for Disease Control and Prevention (CDC)
Division of Select Agents and Toxins (DSAT)
Atlanta, Georgia

United States Department of Agriculture
Animal and Plant Health Inspection Service (APHIS)
Agriculture Select Agent Services (AgSAS)
Riverdale, Maryland

Date: **Month XX, 2016**

Subject: FSAP Policy Statement: Inactivated *Bacillus anthracis*

The Federal Select Agent Program (FSAP) is a collaboration between the Centers for Disease Control and Prevention (CDC), Division of Select Agents and Toxins (DSAT) and the Animal and Plant Health Inspection Service (APHIS) Agriculture Select Agent Services (AgSAS) to regulate the possession, use, and transfer of biological agents listed in 7 C.F.R. Part 331, 9 C.F.R. Part 121, and 42 C.F.R. Part 73 (select agents and toxins). The FSAP administers the select agents and toxins regulations in close coordination with the Federal Bureau of Investigation's Criminal Justice Information Services (CJIS).

Authority:

The U.S. Department of Health and Human Services (HHS) select agents and toxins regulations are found at 42 CFR Part 73. Pursuant to subtitle A of title II of the Public Health Security and Bioterrorism Preparedness and Response Act (the Act), the HHS Secretary has established a list of biological agents and toxins, which have the potential to pose a severe threat to the public health and safety (*See* 42 U.S.C. § 262a). This list is found in section 3 (HHS select agents and toxins) and section 4 (Overlap select agents and toxins) of the HHS select agents and toxins regulations (*See* 42 CFR §§ 73.3, 73.4). Section 3(d)(2) and section 4(d)(2) of the HHS select agents and toxins regulations provide that non-viable select agents are excluded from the HHS select agents and toxins regulations. The Act directs the promulgation of regulations to establish and enforce safety and security procedures for the possession and use of select agents and toxins, including measures to ensure proper training and appropriate skills to handle such select agents and toxins (Ref. 2).

The U.S. Department of Agriculture select agents and toxins regulations are found at 7 CFR Part 331 and 9 CFR Part 121. Pursuant to subtitle B of title II of the Public Health Security and Bioterrorism Preparedness and Response Act (the Agricultural Bioterrorism Protection Act), the USDA Secretary has established a list of biological agents and toxins, which have the potential to pose a severe threat to animal and plant health, or to animal or plant products (*See* 7 USC 8041). This list is found in section 3 (PPQ select agents and toxins) of Part 331 and in section 3 (VS select agents and toxins) and section 4 (Overlap select agents and toxins) of Part 121 of the USDA select agents and toxins regulations. Section 3(d)(2) of Part 331 and sections 3(d)(2) and 4(d)(2) of Part 121 of the USDA select agents and toxins regulations provide that non-viable select agents are excluded from the USDA select agents and toxins regulations. The Agricultural Bioterrorism Act of 2002 also directs the USDA Secretary to promulgate regulations to establish and enforce safety and security procedures for the possession and use of select agents and toxins, including measures to ensure proper training and appropriate skills to handle such select agents and toxins (Ref.3).

Bacillus anthracis, the bacterium that causes anthrax disease, is on the list of overlap select agents and toxins (threat to humans and animals) as a Tier 1 select agent (*See* 42 CFR 73.4 and 9 CFR 121.4). Tier 1 select agents are considered those that have the "greatest risk of deliberate

misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety" (Ref. 4).

Policy Statement:

Although non-viable select agents are excluded from the select agent regulations, it has been observed that some inactivation protocols that have been used have not inactivated *Bacillus anthracis* spores completely, necessitating issuance of this policy statement. Unless waived by the APHIS Administrator or HHS Secretary, it is the policy of the FSAP that all vegetative cell and spore preparations of *Bacillus anthracis* strains regulated as select agents that were subject to an inactivation procedure on or after **June 2, 2015** are considered a select agent and the storage, transfer, or work with this material must comply with regulations found at 42 CFR 73 and 9 CFR 121 until a more effective protocol for inactivation of *B. anthracis* and confirmation of non-viability can be established and validated. This time period was selected based on the date the Federal Select Agent Program (FSAP) issued a moratorium to entities that produces and ships inactivated *B. anthracis* to other laboratories. Possession of such material by an entity not registered to possess the regulated strain of *B. anthracis* or located in a room not listed on a registered entity's registration must be reported within 24 hours of discovery to the FSAP. Reports must be made using the APHIS/CDC Form 3 so that the FSAP may ensure that such material is appropriately destroyed, transferred to a registered entity, or transferred to a registered room.

Inactivated material from strains of *B. anthracis* listed as excluded from the select agent regulations, found at http://www.selectagents.gov/exclusions-overlap.html#bacillus, and specimens presented for diagnosis or verification (section 5(d)) are not subject to this policy.

To submit a waiver of this policy please provide a cover letter describing what material is to be waived, the inactivation protocol and viability test used, and any other supporting information/references.

Strains of *B. anthracis* (vegetative cell or spore preparations) that are described in the following criteria meet the **exclusion** found in section 3(d)(2) and section 4(d)(2) of the select agents and toxins regulations:

1. Preparations that were subjected to an inactivation process or procedure prior to **June 2, 2015,** when FSAP issued through email notification the "Request for immediate moratorium on all work with, and shipments of, inactivated *Bacillus anthracis*."

2. Chemically-treated inactivation procedures that include:
   a. Use of inactivation methods initially validated with kill curves generated by using 100% of the sample.
      1) Use of inactivation contact times that are double the projected zero-growth treatment time derived from the kill curves as an added safety margin.

b. Use of neutralization methods initially validated with neutralization curves by using 100% of the sample and the following positive Control: Split the sample into two portions, and to one, add 10 B. anthracis (Sterne) spores to determine if the chemical (or antimicrobial activity if present) interferes with the viability test.

c. Use of validated viability testing that shows no growth of *B. anthracis* and meets or exceeds the following:
    1) Sample volume: The tested material consists of at least 10% of the sample or 10% of the production lot of inactivated material directly inoculated into a broth medium (e.g., trypticase soy broth, nutrient broth) that supports *B. anthracis* growth. Note: Perform the viability test once chemical and/or antimicrobial treatments have been subjected to a validated neutralizing substance or have been shown not to interfere with the viability test.
    2) Culture conditions: The broth culture is incubated for at least 7 days at 37ºC and then at least 100µl of the broth culture is spread plated to an agar plate medium that supports the growth of *B. anthracis*.
        - The agar plate is incubated at 37ºC for at least 7 days

d. A record is created to document the:
    1) Process or procedures used for chemically treated inactivation,
    2) Results of the final viability testing including the date of those results,
    3) Name of the individual who performed the viability testing, and
    4) Names of recipients of these materials.

3. Chemically-treated whole tissue specimens (such as Formalin fixed tissue)
    a. Use of an inactivation method validated initially to exact conditions to be used for subsequent inactivation.
    b. A record is created to document the:
        1) Process or procedures used for chemically treated whole tissue specimens,
        2) Results of the initial viability testing including the date of those results,
        3) Name of the individual who performed the viability testing, and
        4) Names of recipients of these materials.

4. Untreated preparations with viable agent removed: Vegetative cell and spore free preparations (e.g., nucleic acid extracts, antigens, lysates, etc.) of regulated strains of *B. anthracis* or from material that contains regulated strains of *B. anthracis* (e.g., serum, culture) if the process or procedure meets each of the following requirements:

    a. If the preparation has been produced by a process or procedure that includes filtration through a 0.22 µm or smaller pore size filter , and
    b. Validated viability testing that shows no growth of *B. anthracis* and meets or exceeds the following:
        1) Sample volume: The tested material consists of at least 10% of the sample or 10% of the production lot of inactivated material directly inoculated into a broth medium (e.g., trypticase soy broth, nutrient broth) that supports *B.*

anthracis growth. Note: Perform the viability test once antimicrobial treatments have been subjected to a validated neutralizing substance or have been shown not to interfere with the viability test.

3) Culture conditions: The broth culture is incubated for at least 48 hours at 37ºC and then at least 100µl of the broth culture is spread plated to an agar plate medium that supports the growth of *B. anthracis*.
    - The agar plate is incubated at 37ºC for at least 48 hours.

c. A record is created to document the:
    1. Process or procedures used for vegetative cell-and spore-free preparations,
    2. Results of the final viability testing including the date of those results,
    3. Name of the individual who performed the viability testing, and
    4. Names of recipients of these materials.

5. Autoclaved for use
    a. Use of inactivation methods initially validated with kill curves generated by using 100% of the sample.
    b. Use of autoclave times that are double the projected zero-growth treatment time derived from the kill curves.
    c. Use of validated viability testing that includes the use of an appropriate *Bacillus* species spore based indicator under conditions that accurately represent the types of material that are treated, and shows no growth of *Bacillus* species.
    d. A record is created to document the:
        1. Process or procedures used for autoclave inactivation,
        2. Results of the final viability testing including the date of those results,
        3. Name of the individual who performed the viability testing, and
        4. Names of recipients of these materials.

6. Autoclaved for waste
    Validated viability testing that includes the use of an appropriate *Bacillus* species spore based indicator under conditions that accurately represent the types of material that are treated and shows no growth of *B. anthracis.* This validation should be conducted at least monthly.

Additional information on sterilization methods for waste containing *B. anthracis* can be found in the following references:

1. Whitney, E., Beatty, M., Taylor, T., Weyant, R., Sobel, J., Arduino, M., & Ashford, D. Inactivation of Bacillus anthracis Spores. *Emerg. Infect. Dis. Emerging Infectious Diseases*. 2003; 9(6): 623-627.
2. Rutala, W., Stiegel M., & Sarubbi F. Decontamination of laboratory microbiological waste by steam sterilization. Appl Environ Microbiol. 1982; 43(6): 1311-6.
3. Lauer J., Battles D., & Vesley D. Decontaminating infectious laboratory waste by autoclaving. *Appl Environ Microbiol* 1982; 44(3): 690-4.

4. Wood J., Lemieux P., Betancourt D., Kariher P., & Gatchalian N. Dry thermal resistance of *Bacillus anthracis* (Sterne) spores and spores of other Bacillus species: implications for biological agent destruction via waste incineration**.** *J Appl Microbiol* 2010; 109(1):99-106.
5. United States Pharmacopoeial Chapter (1035). *Biological Indicators for Sterilization*. USP38-NF33, United States Pharmacopoeial Convention, Rockville, MD, 2015.
6. Association for the Advancement of Medical Instrumentation/International Org. for Standardization. *Sterilization of health care products - Biological indicators - Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*. Association for the Advancement of Medical Instrumentation, Arlington, VA, 2006.

References Cited:

1. Simon A. Weller, Margaret G. M. Stokes, Roman A. Lukaszewski. Observations on the Inactivation Efficacy of a MALDI-TOF MS Chemical Extraction Method on Bacillus anthracis Vegetative Cells and Spores. PLoS One. 2015 Dec 3;10(12):e0143870. doi: 10.1371/journal.pone.0143870. eCollection 2015.
2. Public Health Security and Bioterrorism Preparedness and Response Act, 42 U.S.C. § 262a.
3. Agricultural Bioterrorism Act of 2002, 7 U.S.C. § 8401c.
4. Executive Order 13546, "Optimizing the Security of Biological Select Agents and Toxins in the United States," 2010.
5. Dauphin, L., & Bowen, M. A simple method for the rapid removal of Bacillus anthracis spores from DNA preparations. *Journal of Microbiological Methods*. 2009; 76(2): 212-214.

This policy statement will be provided to the Responsible Official via Select Agent (SA) Gram for each registered entity; and to the Responsible Official for each newly registered entity during the registration process. A copy of this policy statement may also be found at http://www.selectagents.gov.

Any question concerning this policy may be addressed by contacting the Federal Select Agent Program at lrsat@cdc.gov or AgSAS@aphis.usda.gov.

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