# Select Agent Investigative Report, Findings and Actions

National Institutes of Health, Bethesda, Maryland

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NIH Investigative Report-Select Agent Program
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**Background**

In November 2011, the National Institutes of Health (NIH) reported the conduct of a "restricted experiment" by a researcher at the Rocky Mountain Laboratories in Hamilton, Montana, a component of the National Institute of Allergy and Infectious Diseases (NIAID), NIH to the Division of Select Agents and Toxins (DSAT), Centers for Disease Control and Prevention (CDC). Upon discovery of this incident, NIH proceeded to take a number of proactive measures to prevent the conduct of this type of experiment elsewhere in the intramural program at the NIH. One of these activities was the retraining of all Select Agent Principal Investigators (PIs) by the NIH Select Agent Program (SAP) Staff in what constitutes a "restricted experiment" under 42 CFR Part 73- Select Agent Regulations.

On January 26 during this re-training effort, two PIs ([redacted]) self-disclosed, to the NIH SAP, work that had taken place from 2002-2005. Literature review revealed that this work was subsequently published in *Infection and Immunity* in January 2006. See Attachment 1. The protocol involved temporarily inserting an antibiotic resistance marker into a selected region of the *B. anthracis* genome and subsequently removing it leaving the target region permanently mutated. One strain thusly manipulated was a pX02+ *B. anthracis* strain; the two antibiotic resistance markers used were erythromycin and spectinomycin. The resulting organism was not resistant to erythromycin but an intermediate organism carried this resistance factor. One such intermediate organism exhibited the capability to produce capsule when incubated in air; scientifically a very interesting event. On January 27, 2012, the NIH SAP reviewed the Select Agent inventory and found that this organism had been added to the inventory in 2005 and has been maintained there to date. No further work has been performed with this organism. On February 2, 2012, NIH received correspondence from DSAT stating that DSAT had determined that it would take no further action in this matter. However, NIH was also advised that any future violations of this type could result in referral to the HHS-Office of Inspector General and in possible civil penalties. This correspondence also requested a summary of the NIH investigative findings in this matter be submitted.

NIH SAP continued to investigate circumstances surrounding the creation of the erythromycin resistant *B. anthracis* strain by [redacted] and co-workers. As part of this investigation, there was a complete search of the 22 refrigerators, freezers and a coldroom used by the laboratory of [redacted] in Building 33 on the NIH Bethesda Campus. [redacted] is a staff scientist in [redacted] laboratory. The contents of refrigerators and freezers located in both BSL-2 and BSL-3 areas used by this laboratory were searched in a "vial by vial" manner. On February 2, 2012, two small vials labeled as follows were found in a freezer in a BSL-2 area (1W equipment corridor) used by this laboratory.

1) A34 ADS11 spores 12/5/05

2) A34 spores heated 30x70 (undated)

These vials belonged to [redacted]. Personnel in this laboratory were not, at this time, registered to possess select agents; the materials were not secured; and these materials had not been identified to the NIH Select Agent Program. The vials were immediately removed from the freezer and transported to the NIH Select Agent Program laboratory where they were entered into the select agent

inventory and secured pending confirmation of the contents of the vials. On February 3, 2012, [redacted] viewed the vials in the NIH SAP laboratory and confirmed that the contents were Ames 34 *Bacillus anthracis* spores. This strain is pX02+ and therefore a regulated select agent. [redacted] stated that they were not aware the vials were present in the freezer in which they were found. DSAT was notified of this finding. See Attachment 2. The two vials of spores were surrendered to the local Weapons of Mass Destruction Coordinator on February 6, 2012. Based on the fact that regulated select agent material was found in unsecured, unregistered space, the NIH SAP, with concurrence of NIH Management, began vial by vial searches of other laboratory and storage areas used by formerly registered *B. anthracis* select agent users to ensure there was no other improperly stored *B. anthracis* in the NIH's possession.

On the evening of Friday February 2, 2012, NIH SAP staff was inspecting the locked freezer of [redacted] in Building 6A Room 2A08. In that freezer a box was found containing 30 unlabeled vials. The box itself was labeled "A-34 all spore". The SAP took possession of the box of vials and entered them into the SAP inventory until the contents could be verified and the situation could be further investigated. On February 6, 2012, it was learned that the contents of the vials were indeed *B. anthracis* spores (pX02+). [redacted] reported that she had "forgot about the spores" until they were found on Friday, February 2nd. It was also discovered at this time that [redacted] laboratory has been using the spores to grow vegetative bacteria for use in opsonophagocytic bactericidal assays and the vegetative bacteria had been grown from the spores on eight trypticase soy agar (TSA) plates as recently as January 2012. [redacted] laboratory had been removed from the NIH Select Agent registration on July 28, 2009. Her material was transferred to the NIH SAP on 10/21/2008 and has been maintained in the NIH SAP inventory since that time. [redacted] also stated that she did not know her laboratory had been removed from the NIH registration. The 30 vials of spores were surrendered to the local Weapons of Mass Destruction Coordinator the morning of February 6, 2012.

Also on February 6, an inspection was begun of the NIH Pilot Plant inventory maintained by [redacted], National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). In his inventory (freezer in Bldg. 14A/ Room 162) 4 vials of *B. anthracis* 6602 (px02+) were found dated 1/23/2001. [redacted] did not recall where these vials came from at the time and there was no documentation immediately available regarding the source of the vials. This strain had not been previously declared to the NIH SAP. No record of use was found for this strain. Building 14A requires card access but the freezer was unsecured in Room 162. [redacted] was removed from the NIH Select Agent Program on 12/18/2003. The NIH SAP is now in possession of these vials and they have been entered into inventory.

On February 6th, the NIH SAP also inspected the freezer in Bldg. 29A/Room 3A19. This room is used by [redacted], Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA). The freezer contained 6 vials of A-34 that were work products of killing assays. [redacted] stated that they should have been destroyed by his technician after the experiment was completed and he did not know they remained in the freezer. Room 3A19 was de-registered at the time the vials were found but was registered for SA work at the time the assays were conducted. The NIH SAP is in possession of all of [redacted] *B. anthracis* stocks and has had them since the laboratory was closed for repairs.

These six "working vials" are now in the possession of the NIH Select Agent program and have been entered into inventory.

On the evening of February 6, 2012, NIH SAP notified DSAT CDC of the three additional incidents described above. See Attachment 3. After these reports were made, NIH received two additional pieces of correspondence from DSAT. See Attachments 4 and 5. The first letter dated February 21, 2012, addressed to [redacted], Select Agent Responsible Official, NIH announced that the unregistered use and storage of *B. anthracis* px02+ strains by [redacted], National Institute of Child Health and Human Development (NICHD), disclosed to DSAT on February 6, 2012, would be investigated by a DSAT team including investigator(s) from the Department of Health and Human Services (HHS), Office of the Inspector General (OIG). The site visit at the NIH, specifically [redacted] laboratories, was announced and subsequently conducted on February 24, 2012. An additional unannounced inspection team returned to the NIH and further investigated the [redacted] matter May 1-4, 2012.

The second letter received from DSAT, dated February 21, 2012, requested the following:

- Weekly updates regarding the NIH progress on inspecting areas where there may be unregistered, nonexempt strains of *B. anthracis* or where evidence leads NIH to believe there might be more pX02+ strains; and
- Weekly updates regarding the NIH investigation into the storage of all exempt strains to ensure that there have been no mistakes in classifying these strains by investigators.

These items were completed on March 2, 2012 and notification was sent to DSAT. See Attachment 6. Further, the letter requested that the NIH Responsible Official provide a complete written report at the conclusion of the NIH investigation, including changes in policies and/or procedures to prevent the storage or use of biological select agents and toxins in nonregistered space. This report is in response to the February 21, 2012 request for a written report and the February 2 request for a summary of findings.

**Investigative Techniques**

The following methods were used to obtain information and investigate the extent to which, if any, unregistered use or storage of Select Agents or the conduct of "restricted experiments" as defined in 42 CFR Part 73 may have occurred at the NIH.

- Select Agent inventory log reviews
- NIH SAP record review
- Review of pertinent e-mail records
- Review of select laboratory notebooks
- Limited literature review
- PI interviews
- Interviews with select laboratory personnel
- Vial by vial audit of all cold storage locations adjacent to any Select Agent storage/use areas

- Inspection of all potential dry storage areas in current or former NIH laboratories storing or working with regulated strains of *B. anthracis*
- Inspection of all potential dry storage areas in current or former NIH laboratories storing or working with exempt *B. anthracis* strains
- Access record review
- Interview of a previous NIAID employee currently working for the FDA

**Findings**

[redacted] and staff members were interviewed on January 30 through February 1, 2012 by [redacted] and [redacted] ([redacted]). These interviews revealed that no one had been working with the erythromycin resistant strain of pX02+ *B. anthracis* or any other pX02+ strain since the laboratory moved to Building 33 in 2007. Given the number of refrigerators and freezers located within this laboratory's assigned space, the RO initiated vial-by-vial searches of all cold locations in the laboratory. Discovery of two vials of pX02+ erythromycin resistant ($Em^R$) *B. anthracis* in an unsecured, hallway freezer led to cold location searches of all PIs currently and formerly registered with the NIH SAP for work with anthrax. The situation was reported to DSAT as previously noted. One other vial was found labeled "A-34" but [redacted] reported it had been mislabeled by a former graduate student, in 2002-2003, in a registered lab in Bldg. 29, and provided the laboratory notebook documentation of this fact. [redacted] was allowed to keep this vial. **Note:** During a subsequent CDC DSAT unannounced inspection (May 1-4 2012) this vial was "re-found" by CDC inspectors who raised further concerns about other vials handled by the same graduate student being mislabeled as A-35 (pX02-) when they actually might be A-34 (pX02+). Given the concern raised by the CDC, the NIH SAP took custody of the vials in question removing them from the [redacted] laboratory. See Attachment 7. In summary, the vials containing pX02+ $Em^R$ *B. anthracis* had not been worked with since 2003. During the move to Bldg 33, the two vials containing the pX02+ $Em^R$ *B. anthracis* were mistakenly stored with unregulated materials and were inadvertently moved to unsecured, unregistered space where they remained, unused, for five years until discovery by the NIH SAP. No one on [redacted] staff has worked with pX02+ strains since moving to Building 33.

Given the findings in [redacted] laboratory, the NIH initiated vial-by-vial searches of all cold storage areas in all laboratories that were currently or previously registered for work with *B. anthracis*.

On February 2, 2012, the Select Agent Program Staff inspected the locked freezer of [redacted] in Building 6, Room 2A08. A box containing 30 unlabeled vials was found in that freezer. The box was labeled "A-34 all spore". The NIH Select Agent Program took possession of the box of vials and entered them into the SAP inventory until the contents could be verified and the situation could be further investigated. On February 6, 2012, [redacted] was interviewed by [redacted], NIH RO, for the first time regarding this matter. [redacted] explained the science behind her work with anthrax and her particular interest in the polyglutamic acid in the capsule of anthrax bacteria. She provided a copy of one of her publications. She said that [redacted], from [redacted] laboratory, was added to her select agent registration and she [redacted] "made the spore prep". This was between July of 2008 and July 2009. [redacted] was appropriately registered for possession and use

of select agents at that time. However, review of records revealed [redacted] made spore preparations in [redacted] lab prior to DSAT approval of the amendment adding her to [redacted] select agent registration. Further, upon review of [redacted] laboratory notebooks, no records could be found for establishing sub-cultures or preparation of spores. There were no notations in the laboratory's select agent inventory log documenting the use of any *Bacillus anthracis* for this purpose. During a subsequent discussion and e-mail exchange with [redacted], on March 22, 2012, it was determined that notations regarding the spore preparation could be found in [redacted] laboratory notebooks. While the spore preps were mentioned in one of the the [redacted] notebooks, there was no information regarding the source or sub-culturing of the *B. anthracis* used in the prep. The spore preparations were made by [redacted] in [redacted] laboratory.

[redacted] Select Agent inventory was removed from her laboratory on October 21, 2008 by the NIH SAP due to security concerns. When asked why she did not relinquish the spores at that time she surrendered the rest of her select agent inventory, said she "didn't think the agent and the spores were the same thing". She later, in the same conversation, said that she "forgot about the spores" until Friday (February 2, 2012) when they were found. She said "I truly thought I gave you everything." She also stated that she was "under the impression" she was still registered for use of select agents. She was asked when she took her annual select agent training and she replied that "we took it in 2008". Pointing out that annual training was required, she was asked why she thought she was still registered after her inventory was removed and no further training had taken place. She replied that she "didn't think of it".

[redacted] was interviewed a second time on February 21, 2012 to obtain further details regarding her relationship with [redacted] and to find out who else may have had access to the spores from 2009 to 2012. [redacted], NIH ARO, was also present during this interview. When asked who had access to the spores she answered that it was only herself, [redacted] and [redacted]. When asked who conducted the assays with the spores she answered that it was [redacted] and [redacted] had also been the one to aliquot the spores into the individual vials and that it was done in room 1A09. When asked where the spores were when the rest of the inventory was removed from her laboratory in October of 2008, she replied that they were kept in '[redacted] freezer" and that she had instructed her to move them to the "anthrax freezer" after the rest of the inventory had been removed. When asked, again, why the spores were not surrendered with the rest of her inventory she answered that when they were "put away for some years, you don't think of it" indicating that regulated anthrax material had been stored in an unregistered, unsecured freezer by [redacted] during the time the laboratory had been registered for use with *B. anthracis*. When asked why the spores were being used in January 2012 she replied that it was for follow-up on the "PNAS paper" thus contradicting earlier statements that she [redacted]) "forgot about the spores" until Friday February 2, 2012. Discussion also took place regarding who else may have been in the area when the spores and bacteria were being handled [redacted] handling of the waste materials (appropriate) and if she had spoken with [redacted] recently (no). In summary, the investigation regarding the spores found in [redacted] unregistered laboratory revealed the following: 1) improper record keeping regarding use of select agents; 2) improper or no inventory records documenting use of these spores for the prep; 3) no record of the spore stock or aliquots in the

laboratory SA inventory; 4) preparation of spores by [redacted] prior to amendment approval by DSAT; 5) improper access control for select agents; 6) failure to follow instructions; 7) failure to relinquish regulated materials to the NIH SAP upon request; 8) subsequent use of those materials; and 9) in general there was a failure to take responsibility for appropriate handling of select agents.

[redacted] was interviewed by [redacted], NIH ARO, on February 6, 2012 and again by [redacted] on February 7th. [redacted] laboratory, Bldg. 29A/Room 3A19 is currently unregistered for select agent storage due to repairs being made to the HVAC system. His select agent inventory was surrendered to the NIH SAP on 11/24/2009 for the duration of the renovation, recertification, and DSAT re-registration process. [redacted] was absolutely positive he had no select agents in his possession and accompanied [redacted] during the vial-by-vial check of his cold storage locations. The freezer in Room 3A19 contained 6 vials labeled A-34 that were work products of killing assays. [redacted] stated that they should have been destroyed by his technician after the experiment was completed and he did not know they remained in the freezer. The freezer in which the vials were found was locked and located within a BSL-3 laboratory with biometric access granted only to those individuals who were cleared to work with SA when the laboratory was registered to use SA. No other improperly stored regulated materials were found.

[redacted] was interviewed by [redacted] and [redacted], NIH SAP AROs, on February 6, 2012. Review of detailed inventory records revealed that un-registered *Bacillus anthracis* may be present in his pilot plant inventory. In his inventory, contained in the freezer in Bldg. 14A/ Room 162, 4 vials *of B. anthracis* 6602 (px02+) were found dated 1/23/2001. At the time, [redacted] did not recall where these vials came from and there was no documentation immediately available regarding the source of the vials. This strain had not been previously declared to the NIH SAP. No record of use was found for this strain in the very detailed pilot plant production records. [redacted] was removed from the NIH Select Agent Program on 12/18/2003. Vial-by-vial inspection of the pilot plant freezer did not result in discovery of any other select agents.

Given the unexpected results of the vial-by-vial searches in the laboratories of these four PIs, all of whom worked in different Institutes/Centers (ICs) of the NIH, NIH management agreed that the cold storage areas of any PI working with select agents, currently or previously, would be subjected to vial-by-vial inspection. Also, included in the audit were PIs known to work with unregulated strains of *B. anthracis* (pX02-). Three hundred and thirty cold storage areas (freezers, refrigerators, cold rooms, liquid nitrogen freezers) used by 20 PIs were inspected on the NIH campus and at the Integrated Research Facility, National InterAgency Biodefense Campus, Ft. Detrick, Maryland. It is estimated that approximately 6 million vials were checked during this time. The vial-by-vial audit was completed on April 13, 2012.

The CDC DSAT also requested that all possible dry storage areas (cabinets, drawers, desks, etc.) in the Laboratory of [redacted] be searched. This was completed in April 4/17/2012.

No further inappropriately stored select agent materials were found during the cold and dry location audits.

In order to prevent any further storage or use of biological select agents and toxins in nonregistered spaces at the NIH, the following actions have been taken.

**Administrative Actions Taken**

1) NIH SAP identified the need to expand documentation beyond that required by the Select Agent regulations. Procedures have been changed to include specific documentation to every Select Agent PI/Supervisor when an individual has been removed from the program or their access has been removed. See Attachment 8 and 9. This was instituted on February 7, 2012.
2) NIH SAP procedures have been modified to ensure that prior to de-registering space, storage areas do not contain "remnant" select agent materials. See Attachment 10. This was instituted February 7, 2012.
3) NIH SAP identified the need for PIs to agree, in writing, to their responsibility for the select agents in their possession from "cradle to grave". See Attachment 11. This was instituted February 7, 2012.
4) The NIH SAP has requested a complete accounting of everyone who has been issued a "hard" key to a select agent controlled space, from the NIH Locksmith, to ensure that only authorized personnel have access to these areas.
5) Select Agent training materials have been modified to further emphasize accountability, responsibility and the penalties associated with failure to adhere to select agent requirements.
6) The Scientific Director for the National Institute of Child Health and Human Development (NICHD) has suspended a non-compliant laboratory's privilege to conduct work with any human pathogen in addition to select agents.
7) Formal disciplinary actions have been proposed by the NIH, where warranted, and are in process.

**Management Actions Taken**

The NIH Deputy Director for Intramural Research (DDIR) required that each PI of a laboratory attest, in writing, to the fact that they have surveyed their laboratory spaces for select agent materials and that none were found. If unregistered materials were found, the PI was required to contact the appropriate RO for the NIH location. See Attachment 11. Approximately 1200 PIs from NIH ICs that conduct intramural laboratory research, on any NIH campus, completed these written attestations. The Scientific Director of each IC was responsible for ensuring that all PIs complied with this requirement. The completed attestation documents were reviewed by [redacted] staff members. No select agents were identified. Exempt quantities of toxins in various locations were identified and appropriate follow-up was provided to ensure these materials are registered with the Division of Occupational Health and Safety, NIH.

The Scientific Director, National Institute of Allergy and Infectious Diseases (NIAID) is adding a "Select Agent Compliance" element in Performance Management Appraisal Program (PMAP) Plans of all PIs working with Select Agents.

The NIH Deputy Director for Management (DDM) initiated review of the NIH Table of Penalties (TOP) with regard to its applicability to violations of the Select Agent regulations or NIH policies governing use of these agents. This review resulted in revision of the TOP to specifically address Select Agents. The revision referencing select agents was implemented on May 7, 2012 and is included below.

## Select Agents and Toxins

| NATURE OF MISCONDUCT | FIRST ACTION | SECOND ACTION | THIRD ACTION |
|---|---|---|---|
| A SINGLE ACT OF GROSS NEGLIGENCE CAN WARRANT REMOVAL FOR A FIRST OFFENSE. | | | |
| -PROCEDURAL OR ADMNISTRATIVE DEVIATION FROM PROCEDURES FOR THE POSSESSION, USE, OR TRANSFER OF SELECT AGENTS OR TOXINS OR OTHER REGULATED MATERIALS | REPRIMAND TO REMOVAL | 14-DAY SUSPENSION TO REMOVAL | REMOVAL |
| -FAILURE TO PROPERLY SAFEGUARD OR SECURE SELECT AGENTS OR TOXINS OR OTHER REGULATED MATERIALS | REPRIMAND TO REMOVAL | 14-DAY SUSPENSION TO REMOVAL | REMOVAL |
| -FAILURE TO PROPERLY DISPOSE SELECT AGENTS OR TOXINS OR OTHER REGULATED MATERIALS | REPRIMAND TO REMOVAL | 14-DAY SUSPENSION TO REMOVAL | REMOVAL |
| **Note: * Biological agents and toxins that could pose a severe threat to public health and safety, to animal health, or to animal products.** | | | |

* As defined by 42 Code of Federal Regulations (CFR), Part 73; 9 CFR, Part 121; and 7 CFR, Part 331

### Conclusion

Upon discovery of inappropriate storage and use of select agents, the NIH notified the CDC Division of Select Agent and Toxins and then rapidly and proactively embarked upon an extensive investigation to ensure that no other regulated select agent materials were inappropriately handled or stored at the NIH. The findings of this investigation resulted in immediate changes to NIH SAP procedures; administrative actions including proposals of disciplinary action; and management actions resulting in NIH-wide activities to further ensure appropriate use and storage of select agents at the NIH.