(Rev. 08-28-2000)



# FEDERAL BUREAU OF INVESTIGATION

**Precedence:** ROUTINE **Date:** 11/01/2005

**To:** Counterterrorism **Attn:** WMDOU

Inspection **Attn:** IIC [redacted]

Washington Field **Attn:** SSA [redacted]
SSA
A/SSA [redacted]

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**From:** Washington Field
Amerithrax-2
**Contact:** SA [redacted]

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**Approved By:** [redacted]

**Drafted By:** [redacted]

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**Case ID #:** 279A-WF-222936-DUGWAY (Pending) ✓ 7

**Title:** AMERITHRAX;
MAJOR CASE 184

**Synopsis:** To summarize the investigations involving the laboratory notebook of [redacted] U.S. Army Dugway Proving Ground, initiated on 04/07/1997 in response to Dr. Bruce Ivins' request for anthrax spore preparation using bench top fermentors.

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**Enclosure(s):** Three page summary diagram of productions runs that were used to compile RMR 1029.

**Reference:** 279A-WF-222936 Sub USAMRIID Serial #795
279A-WF-222936 Sub USAMRIID Serial #882
279A-WF-222936 Sub DPGBA Serial #035

**Details:** In a communication set forth by Dr. Bruce Ivins, U.S. Army Medical Research Institute of Infectious Disease, USAMRIID, on 01/17/1997, entitled "SPORES, SPORES, SPORES," Ivins explained that it took 13 anthrax spore preparation runs to produce a total of $3.0 \times 10^{12}$ total spores (approximately three (3) grams of dried, purified spores). Ivins went on to explain that for upcoming animal challenge experiments, 10 times this amount of spores would be needed. Subsequently [redacted] was contracted

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to produce spores for Ivins under the project entitled "Procedure for Anthrax Spore Preparation in Bench Top Fermentors." b6 b7C

On 03/25/1997, [redacted] received four (4) 1ml polypropylene tubes containing *Bacillus anthracis* Ames for use in fermentor production runs. On 04/07/1997 [redacted] began the first spore production run of the 19 total runs that would be initiated for shipment to Ivins/USAMRIID between 04/23/1997 and 09/03/1997.

**Summary of Fermentation Production Runs**

On 04/12/1997, [redacted] completed the first fermentation production run, which resulted in **$6.3x10^{12}$ total spores** or 70ml of $9.0x10^{10}$ spores/ml. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 04/14/1997, [redacted] completed the second fermentation production run, which resulted in **$9.5x10^{12}$ total spores** or 150ml of $6.3x10^{10}$ spores/ml. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 04/18/1997, [redacted] **aborted** the fermentation run that began on 04/15/1997 due to *Bacillus globigii* contamination. According to [redacted] notebook this batch was autoclaved on 04/18/1997.

On 04/22/1997, [redacted] completed the fourth fermentation production run, which resulted in **$7.0x10^{12}$ total spores** or 280ml of $2.5x10^{10}$ spores/ml. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 04/25/1997, [redacted] completed the fifth fermentation production run, which resulted in **$7.5x10^{12}$ total spores** or 250ml of $3.0x10^{10}$ spores/ml. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 05/19/1997, [redacted] completed the sixth fermentation production run, which resulted in **$4.8x10^{12}$ total spores** or 120ml of $4.0x10^{10}$ spores/ml. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 05/22/1997, [redacted] completed the seventh fermentation production run, which resulted in **$5.3x10^{12}$ total spores** or 155ml of $3.4x10^{10}$ spores/ml. During dilutions both

before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 06/23/1997, [redacted] **aborted** the fermentation run that began on 06/19/1997 due to *Bacillus globigii* contamination. According to [redacted] notebook this batch was autoclaved (contamination and autoclave notation was not dated, but appeared under the 06/23/1997 entry). b6 b7C

On 06/28/1997, [redacted] completed the ninth fermentation production run, which resulted in **$7.3x10^{12}$ total spores** or 125ml of $5.8x10^{10}$ spores/ml. During dilutions titers of raw spores two (2) colonies of *Bacillus globigii* were noted on a single plate of nearly confluent *Bacillus anthracis*. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 07/01/1997, [redacted] **aborted** the fermentation run that began on 06/28/1997 due to *Bacillus globigii* contamination. According to [redacted] notebook this batch was autoclaved on 07/01/1997.

On 07/11/1997, [redacted] completed the eleventh fermentation production run, which resulted in a reported **$5.34x10^{12}$ total spores** or 175ml of $3.6x10^{10}$ spores/ml. Calculations using 175mls at $3.6x10^{10}$ spores/ml are actually **$6.3x10^{12}$ total spores**. During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 07/17/1997, [redacted] completed the twelfth fermentation production run, which resulted in reported **$5.2x10^{12}$ total spores** or 325ml of $1.7x10^{10}$ spores/ml. Calculations using 325mls at $1.7x10^{10}$ spores/ml are actually **$5.5x10^{12}$ total spores.** During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 07/21/1997, [redacted] completed the thirteenth fermentation production run, which resulted in 250ml of $2.0x10^{10}$ spores/ml (approximately **$5.0x10^{12}$ total spores**). During dilutions after heat shock procedures one (1) colony of *Bacillus globigii* contamination was noted on one (1) dilution plate.

On 07/29/1997, [redacted] **aborted** the fermentation run that began on 07/24/1997 due to *Bacillus globigii* contamination. According to [redacted] notebook this batch was autoclaved (contamination and autoclave notation was not dated, but appeared under the 07/29/1997 entry).

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On 08/02/1997, [redacted] completed the fifteenth fermentation production run, which resulted in 200ml of $3.4x10^{10}$ spores/ml (approximately **$6.8x10^{12}$ total spores**). During dilutions both before and after heat shock procedures no *Bacillus globigii* contamination was noted.

On 08/04/1997, [redacted] completed the sixteenth fermentation production run, which resulted in 180ml of $3.1x10^{10}$ spores/ml (approximately **$5.6x10^{12}$ total spores**). During dilutions before heat shock procedures three (3) colonies of *Bacillus globigii* contamination were noted on one (1) dilution plate.

On 09/09/1997, [redacted] completed the seventeenth fermentation production run, which resulted in 170ml of $4.6x10^{10}$ spores/ml (approximately **$7.8x10^{12}$ total spores**). During dilutions before heat shock one (1) colony of *Bacillus globigii* contamination was noted on one (1) dilution plate at two (2) separate dilutions, and after heat shock procedures one (1) colony of *Bacillus globigii* contamination was noted on one (1) dilution plate.

On 09/15/1997, [redacted] completed the eighteenth fermentation production run, which resulted in 180ml of $2.7x10^{10}$ spores/ml (approximately **$4.9x10^{12}$ total spores**). During dilutions after heat shock procedures three (3) colonies of *Bacillus globigii* contamination were noted on one (1) dilution plate.

On 09/23/1997, [redacted] completed the nineteenth fermentation production run, which resulted in an unknown quantity and an unknown concentration. No *Bacillus globigii* contamination was noted during the concentration assay. The disposition of this fermentation run is unknown, however, 70ml of irradiated spores, concentration $5.0x10^{7}$ spores/ml, dated 09/23/1997 were recovered in a consensual search of the Lothar Salomon Life Sciences Test Facility at the Dugway Proving Ground, Dugway, Utah on 06/30/2004.

**Summary of *Bacillus globigii* Contamination**

*Bacillus globigii* contamination was noted in five productions runs dated 06/28/1997, 07/21/1997, 08/04/1997, 09/09/1997, and 09/15/1997. All five (5)of these runs were sent to Ivins at USAMRIID of which three (3) were added to a stockpile compiled by Ivins call RMR 1029. Additionally, four (4) productions runs, (04/18/1997, 06/23/1997, 07/01/1997, 07/29/1997) were aborted and autoclaved due to *Bacillus globigii* contamination. There is no explanation in [redacted] notes as to a

threshold for *Bacillus globigii* contamination of which, if exceeded, should call for aborting and autoclaving the production.

Summary of Samples Recovered

Samples labeled with dates consistent with production runs form the project entitled "Procedure for Anthrax Spore Preparation in Bench Top Fermentors" were recovered in a consensual search of the Lothar Salomon Life Sciences Test Facility at the Dugway Proving Ground, Dugway, Utah on 06/30/2004. Irradiated samples bearing dates 04/12/1997 (0.5ml), 04/14/1997 (0.5ml), 04/22/1997 (0.3ml), 05/22/1997 (0.25ml), 07/11/1997 (0.25ml), 07/17/1997 (0.5ml), and 07/21/1997 (0.25ml) were all recovered from the above search. One (1) sample bearing the date 08/03/1997 (0.25ml) was also recovered in this search, however, this date falls directly between production runs from 08/02 and 08/04, and therefore may or may not be correlated with the material shipped to USAMRIID for this project.

Additionally, 19 scanning electron microscopy(SEM) stubs were also collected during this search. Ten (10) of SEM the stubs can be associated by dates with production runs from this Dugway/USAMRIID project. SEM stubs correlated with production dates 06/28/1997 and 08/04/1997 were not recovered. All SEM stubs that were recovered and believed to be associated with this production project were analyzed using SEM/energy dispersive x-ray spectroscopy (EDS) by the FBI laboratory's Chemistry Unit, Quantico, Virginia. No silicon, tin, or iron signatures were identified in these specimens.

Of the material recovered from these searches, no live spore material bearing production dates from this Dugway/USAMRIID project was reclaimed.

It should be noted that there are erroneous calculations and inconsistencies in volumes when comparing [redacted] notebook with the Quality Assurance/ Quality Control paperwork affiliated with these production runs. Understanding these inconsistencies along with the level of *Bacillus globigii* contamination in materials shipped from Dugway to USAMRIID is critical for furthering investigation into genotypically compelling anthrax materials at USAMRIID. Based on genetic and forensic signatures pertaining to the RMR 1029 spore material, further exchange with Amerithrax investigators and [redacted] may be warranted.

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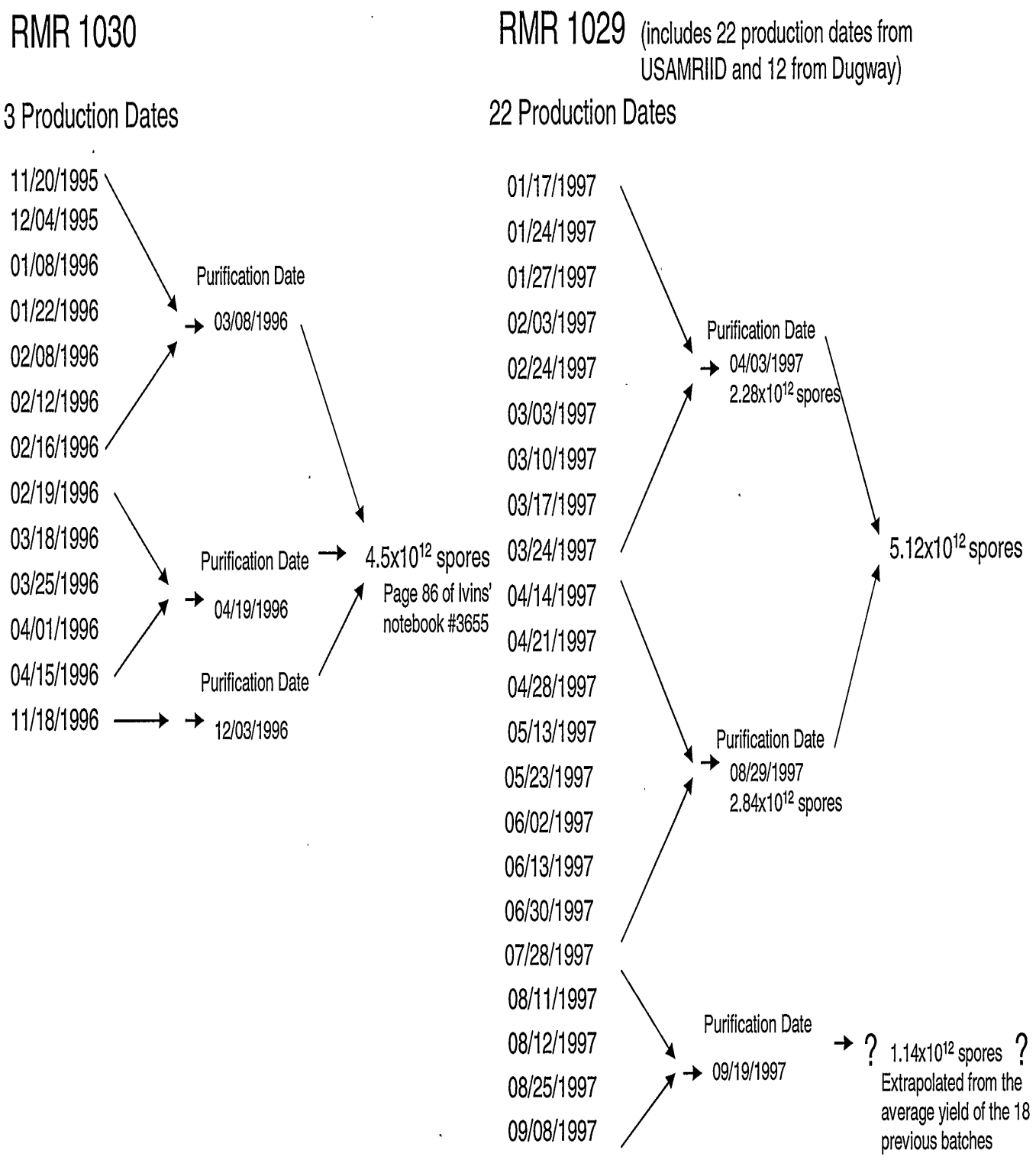


# USAMRIID Anthracis Production of RMR 1030 and 1029

# RMR 1029

## 12 Production Dates

| | | | | | | |
|---|---|---|---|---|---|---|
| Shipped on 04/23/1997 | $15.80 \times 10^{12}$ spores | **04/12/1997**<br>**04/14/1997** | Received on 04/24/1997 | Purification Date 04/28/1997 | $3.9 \times 10^{12}$ (75.32% purification loss) (First two lots described as contaminated) | |
| Shipped on 06/24/1997 | $19.03 \times 10^{12}$ spores | **04/22/1997**<br>**04/25/1997**<br>05/19/1997 | Received on 06/25/1997 | Purification Date 07/14/1997 | $6.5 \times 10^{12}$ (66.32% purification loss) | |
| Shipped on 07/09/1997 | $12.57 \times 10^{12}$ spores | **05/22/1997**<br>*06/28/1997* | Received on 07/10/1997 | Purification Date 07/23/1997 | $8.53 \times 10^{12}$ (32.14% purification loss) | |
| Shipped on 07/23/1997 | $10.57 \times 10^{12}$ spores | **07/11/1997**<br>**07/17/1997** | Received on 07/24/1997 | Purification Date 08/29/1997 | $7.34 \times 10^{12}$ (30.36% purification loss) | |
| Shipped on 08/06/1997 | $09.08 \times 10^{12}$ spores | ***07/21/1997***<br>08/02/1997 | Received on 08/07/1997 | Purification Date 09/01/1997 | $3.89 \times 10^{12}$ (57.16% purification loss) | |
| Shipped on 09/03/1997 | $05.58 \times 10^{12}$ spores | *08/04/1997* | Received on 09/04/1997 | Purification Date 09/16/1997 | $3.8 \times 10^{12}$ (31.90% purification loss) | |
| Total $72.87 \times 10^{12}$ spores | | | | | Total $33.96 \times 10^{12}$ spores | |

Combining production runs from USAMRIID and Dugway yielded approximately **$4.02 \times 10^{13}$ spores** (reported by Ivins as $3.6 \times 10^{13}$ spores) without the 7th lot.

(*Positive for *Bacillus globigii* colonies during dilutions *
Bold **dates** correlate with samples recovered from AMX search of the Life Sciences Facility)

Dugway Proving Grounds Anthracis Production of 1029

# Dugway's 7th Lot

Shipped on 09/24/1997 → 12.68x$10^{12}$ spores ← *09/09/1997* / *09/15/1997* ← Received on 09/25/1997 → Purification Date 10/01/1997 → Not added to RMR 1029

According to QA/QC volumes and concentrates

*Positive for *Bacillus globigii* colonies during dilutions *

## Additional Dugway Production Dates:

4/18/1997 → *Bacillus globigii* contamination noted, production aborted and autoclaved

6/23/1997 → *Bacillus globigii* contamination noted, production aborted and autoclaved

7/01/1997 → *Bacillus globigii* contamination noted, production aborted and autoclaved

7/29/1997 → *Bacillus globigii* contamination noted, production aborted and autoclaved

FD-302 (Rev. 10-6-95)



**FEDERAL BUREAU OF INVESTIGATION**

Date of transcription 12/08/2006

On December 7, 2006, [redacted] U.S. Army, Dugway Proving Ground, Dugway, Utah 84022, telephone number: [redacted] date of birth: [redacted] SSN: [redacted] was interviewed at Washington Field Office, 601 4th Street, N.W., Washington, DC. After being advised of the identity of the interviewing agents and the purpose of the interview, [redacted] provided the following information: b6 b7C

The events of September 11, 2001 on Dugway personnel was effected four fold. The general route that Life Sciences employees take into work was changed, rerouting individuals away from the Chemical laboratory for safety purposes. Fences and checkpoints were erected. There was an increase in the overall number of guards on base. And, scrutiny of individuals coming and going from the facility increased. Although [redacted] could not recollect, [redacted] suggested that September was historically a good time of year for field trials, which would require access to the laboratory during off hours. [redacted] does not recall a non-essential personnel lock-down of Dugway facilities following the attacks of September 11, 2001.

Subsequent to the FBI's inquiry into access of their Biosafety Level-3 (BL-3) facility and general anthrax letter inquiries, plans for increasing security over the biological program facility were fashioned. The new security was to replicate or overlay the Chemical Program assurity plan.

[redacted] explained that the [redacted], Building [redacted] was basically an abandoned building in 2001, had limited power, and limited to basic locking mechanisms in place. [redacted] added that Building [redacted] at that time could have had operational fermentor/culturing capabilities for *Bacillus globigii* (*Bg*)- (easily identified and characterized by its custard orange colonies) and possibly *Bacillus thuringiensis* (*Bt*), but not for *Bacillus anthracis* (*Ba*). [redacted] were part of the [redacted] who used Building [redacted] b6 b7C b7F

Investigation on 12/07/2006 at Washington, D.C.

File # 279A-WF-222936-DUGWAY - 10 Date dictated 12/08/2006

by SA [redacted] SA b6 b7C

German Village, was a facility used for training exercises. This facility used only mock equipment and was non-functional

[redacted] explained that DR. BRUCE IVINS, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), in the past, has sent seed stock on two to three occasions. [redacted] confirmed through viewing shipping records from 1992 that the first transfer of AMES to Dugway was in October, 1992. This material was transferred in two 15mL polypropylene screw capped tubes. [redacted] confirmed through viewing shipping records from 1997 that second transfer from USAMRIID to Dugway was in March, 1997. This transfer included four one (1)mL vials of Ames spores.

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[redacted] described two major Dugway growth projects aside from the 1997 Dugway-USAMRIID project that took place in the 1990's as the Chemical Biological Mass Spectrometer (CBMS) project and the Battelle, High Temperature Incendiary project.

The CBMS project took place approximately from 1994-1996, used a host of different strains of *Ba* (Zimbabwe, Vollum, Sterne, and Ames) as well as *Yersinia pestis* and *Francisella tularensis*. In the case of Ba, Casein Acid Digest (CAD) and Leighton-Doi (media) was used to grow (in fermentors) and harvest cells from log phase as well as spores from stationary phase. These products were then sent to USAMRIID as a concentrated slurry and irradiated by either [redacted] or [redacted] Dugway upon receipt of irradiated spores lyophilized (dried) the spores. [redacted] does not recall drying irradiated AMES for this project. [redacted] identified [redacted] as the [redacted] turned over to by [redacted] In viewing various scanning electron microscopy (SEM) images related to this CBMS project, [redacted] explained that the Standard Operating Procedure (SOP) for this CBMS project required for quality assurance/quality control (QA/QC) measures in the form of SEM images from each growth batch be taken.

The Battelle, High Temperature Incendiary Project utilized only the Vollum strain of *Ba*. Spores from this project were acetone dried. This method was taught to [redacted] by [redacted] in 1998.

Shown pictures of vials recovered from a FBI conducted consent search in 2004, [redacted] identified picture labeled #72-74 as the container within the can which contained the four (4)

vials of seed stock originally sent by IVINS in 1997. [redacted] identifies picture #72 as beads [redacted] made from IVINS' original spore stock in November, 2003 for another Dugway-USAMRIID bulk spore growth project. [redacted] identifies the tube in picture #74 labeled "B. ant Ames" as an original March, 1997 seed stock tube ambiguously labeled by IVINS. [redacted] additionally identified the tubes in pictures #73 and 75 as identically labeled March 1997, IVINS seed stock.

[redacted] described the process by which [redacted] produced the beads described in picture #72 as streaking spores for isolation, picking 3-5 colonies, and adding them to the bead storage solution.

[redacted] confirmed that only one tube of this original stock was used in the 1997 production runs that contributed to RMR 1029. [redacted] explained that [redacted] would steak for isolation on either Blood Agar Plates (BAPs) or Tryptic Soy Agar (TSA), pick multiple, three (3) to five (5), colonies, resuspend and used to inoculate BAPs for confluent growth. BAPs were then used to inoculate the Leighton-Doi media contained in a glass fermentation vessel. [redacted] always picks more than one colony for inoculation in order to assure a heterogenous population. [redacted] added Antifoam A to the media before sterilization. [redacted] did not control for pH during fermentation of Ba. [redacted] believes that controlling for pH is unnecessary in that a natural rise (basic), fall (acidic), and rise (basic) profile is a natural process in the cellular life cycle.

[redacted] described the *Bg* contamination of 1997 Dugway-USAMRIID batches as either environmental contamination of the count plates, post fermentation (suitable to send on to USAMRIID), or contamination within the fermentation (not suitable to send on to USAMRIID). Unsuitable fermentation runs were destroyed by autoclaving. [redacted] described the noted 2003 *Bacillus subtilis* (*Bs*) contamination as one in the same, or interchangeable, with the *Bg* contamination describe earlier as easily distinguished custard orange colonies. [redacted] has never noted a mucoid/contamination colony in any fermentation run.

[redacted] was unable to explain, based on [redacted] notebook entries and QA/QC testing any difference between fermentation production runs dated 09/09/1997 and 09/15/1997 (the 7th lot) which were described by IVINS to be "too dirty" to be added to RMR 1029. [redacted] proceeded with one additional fermentation run

on 09/23/1997, but did not ship this to USAMRIID based on the fact that [redacted] had already shipped more than enough product to fulfill the original contract.

[redacted] theorized that a batch or spores stored in water for a long period of time can seep DNA and become "gooey," and unable to be cleaned. However, the fact the 7th lot was immediately purified by IVINS without unnecessary storage and the fact that spores produced for this project were still utilizable after several years made this "DNA seepage" scenario unlikely in [redacted] opinion.

Based on detailed listings of the 2004 consent search collected items [redacted] identified the SEM stubs as well as the "EM specimens," bearing the same dates as 1997 production runs, as QA/QC derived samples and SEM stubs from the 1997 production runs.

[redacted] affirmed that there were mistakes in additive (salts) concentrations in the August of 1997 SOP, generated upon completion of the 1997 spore production project. [redacted] confirmed that the concentration for $FeSO_4$ was higher than the concentration called for in the original Leighton-Doi protocol. [redacted] also confirmed that this mistake may have carried over into the latter 1997 production runs.

[redacted] believes that shipping records for AMES samples at Dugway's Life Sciences Division are accurate in that there are no additional unknown or undocumented AMES shipments.

[redacted] did not recall any 2004 envelope aerosolization study at Dugway, and therefore could not provide a client or Principle investigator for this project.

[redacted] describes contractor clearances to work in the [redacted] suite at Dugway as needing a secret clearance, having completed the personal reliablility program (PRP), having completed a mentoring program, and having the proper vaccinations. Contractors were escorted within the [redacted] suite.

[redacted]

[redacted]

[redacted] described [redacted] as an eager to please oddball and a capable scientist with fermentation experience. [redacted] described [redacted] as a reliable old timer and a capable scientist with little fermentation experience.

[redacted] provided a list of contact information for [redacted] personnel. This list, the original interview notes, and a packet of visual reference materials used during the interview will be kept in the FD-340 section of the file, Serial 1A-7087.