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FEDERAL BUREAU OF INVESTIGATION

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Precedence: ROUTINE **Date:** 05/24/2005

To: Inspection **Attn:** [redacted]

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

From: Washington Field Office
Amerithrax-3
Contact: SA [redacted]

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-15-2008 BY 60324 UC BAW/DK/TH

Approved By: [redacted]

Drafted By: [redacted]

Case ID #: 279A-WF-222936-USAMRIID (Pending) -1309

Title: AMERITHRAX;
MAJOR CASE 184

[redacted]

Synopsis: To summarize the events surrounding the unauthorized environmental surveys conducted by BRUCE IVINS at USAMRIID in December 2001 and April 2002, and to provide an assessment of IVINS' stated motivations and documented actions.

Details: Following is a synopsis of the events surrounding the unauthorized environmental surveys conducted by BRUCE IVINS at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in December 2001 and April 2002. IVINS' surveys occurred after the anthrax-laced letters mailed in September and October of 2001 were received at USAMRIID for examination. In the aftermath of IVINS' April 2002 survey, multiple investigations were initiated. The U.S. Army launched both Army Regulation 15-6 (AR 15-6) and Criminal Investigation Command (CID) investigations. Additionally, the FBI conducted an independent query into the finding of *Bacillus anthracis* (*B.a.*) contamination in "cold" (non-containment) areas of USAMRIID pursuant to the AMERITHRAX investigation.

The overriding purpose of the compilation and summary contained herein was to provide a rigorous evaluation of IVINS' stated motivations and documented actions in relation to the unauthorized sampling missions. Information from the AR 15-6, CID and FBI investigations was compiled in order to construct a comprehensive summary of the events leading up to and in response to IVINS' survey results. Interviews and sworn statements

[redacted]

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derived from multiple USAMRIID employees were assessed for details related to IVINS' actions, statements and motivations. The individuals referenced herein were all USAMRIID employees at the time of the environmental surveys. [redacted] were provided in response to Federal Grand Jury subpoena #1228. b3

Writer's comments, enclosed in brackets and written in italics, have been included throughout the summary. Comments include observations regarding behaviors of USAMRIID employees, notable supportive and contradictory information, comparisons of results from multiple environmental surveys, and speculations regarding possible alternative explanations for IVINS' actions. The writer's comments are topics to be addressed during pending interviews.

EVENTS PRECEDING IVINS' DECEMBER 2001 INDEPENDENT ENVIRONMENTAL SURVEY

- *Concern regarding safe handling of B.a.*

According to [redacted] USAMRIID received the Daschle letter for analysis on either October 15 or 16, 2001.[1] The letter was initially processed in [redacted] and saw [redacted] exit [redacted] wearing one glove and carrying a slide in a small ziplock big. [redacted] was concerned about contamination because [redacted] had assumed that [redacted] had forgotten to take off the glove prior to exiting the laboratory to the outside hallway.[2] [redacted] shared this concern with BRUCE IVINS [redacted] samples were being processed initially in a BioSafety Level 2 (BSL-2) laboratory. According to [redacted] did not raise this concern as a huge issue. Additionally, [redacted] was not scared, nor did [redacted] feel that other people's safety was threatened.[4] b6 b7C

During interviews with the FBI, IVINS [redacted] stated independently that in the fall of 2001, after USAMRIID received the Daschle letter tainted with *B.a.*, [redacted] concern that some of the handling procedures in the Diagnostic Systems Division (DSD) appeared to be unsafe.[5] According to IVINS, [redacted] repeatedly voiced reservations regarding the way that incoming samples were handled during [redacted] [redacted] told IVINS [redacted] on several occasions that safety and contamination requirements were not being met by [redacted] personnel who were inventorying and examining evidence.[7] [redacted] was unsure about the specifics of [redacted] comments, but indicated that [redacted] statements occurred during normal office conversation.[8] To [redacted] it was not clear b6 b7C

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whether procedures were really not being performed safely, or whether procedures were just being conducted differently than [redacted] had seen previously, and [redacted] interpreted the differences to be unsafe.[9]

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Sometime soon after the glove incident occurred, [redacted] was familiar with the incident, [redacted] the glove was never in the suite, rather it was [redacted] a small tube of liquid that accompanied the Daschle letter to carry to another laboratory. The tube was double bagged in ziplocks and the exterior was bleached.[10] [redacted] felt comfortable with the fact that there were no contamination concerns. [redacted] was not absolutely certain, but [redacted] relayed to IVINS [redacted] that [redacted] original concern about unsafe handling of evidence was no longer a concern.[11]

[WRITER'S COMMENTS - Relative to IVINS, [redacted] described very different reactions to [redacted] safety concerns. During interviews and in sworn statements, [redacted] minimized the discussions of and incidents related to safety concerns, while IVINS inflated them. [redacted] referenced one or a few discussions, while IVINS indicated that [redacted] had multiple, "daily" discussions regarding safety concerns. [redacted] indicated during an interview with the FBI that [redacted] was no longer concerned with contamination issues following [redacted] discussion with [redacted] referenced the glove incident on multiple occasions during interviews and sworn statements. Neither IVINS [redacted] mentioned that [redacted] informed them of [redacted] discussion with [redacted] or that [redacted] conveyed to [redacted] that [redacted] was no longer concerned.]

- *Handling of the Daschle letter in suite B3 by IVINS and [redacted]*

In the fall of 2001, soon after receipt of the Daschle letter at USAMRIID, IVINS [redacted] conducted preliminary work with the *B.a.* evidence.[12] IVINS was tasked with assessing the concentration of the anthrax powder in the Daschle letter.[13] IVINS entered the evidence into USAMRIID's suite B3, a BSL-3 laboratory, through the B3 pass-thru box.[14] IVINS stated that the contents of the letter contained a powder that was unlike anything he had ever dealt with previously. Upon examination, the powder contained in the letter seemed to float easily in the air.[15] IVINS was surprised by the fineness of the powder and stated that "it floated around inside the hood like dust in the sunlight."[16] IVINS stated that due to the ease at which the

[redacted]

powder became airborne, USAMRIID personnel should have conducted testing within a laboratory that bore an overheard containment hood.[17]

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IVINS' DECEMBER 2001 INDEPENDENT ENVIRONMENTAL SURVEY

- *IVINS' motivations for conducting an unauthorized environmental survey*

IVINS became concerned that the fine powder of the Daschle letter, as well as other potential anthrax letters and samples, were not contained adequately with the practices used at USAMRIID.[18] IVINS disagreed with [redacted] processing of the Daschle letter in Room [redacted] as the BSL-2 laboratory was not adequate to contain aerosolized *B.a.* powder.[19] According to IVINS, [redacted] commented "daily" to him that [redacted] employee laboratory practices were unsafe.[20] Based on conversations with [redacted] IVINS became concerned about possible contamination outside the actual laboratories where the evidence was being handled. IVINS was also apprehensive that contamination of personnel might lead to contamination of areas outside the containment suites. [redacted] mentioned to IVINS the concern that [redacted] may have been exposed to powder and possibly contaminated.[21] Over time, IVINS' concern extended to the office area which [redacted] He felt that if there had been unsafe handling of evidence, then [redacted] could have inadvertently contaminated the office.

In December 2001, IVINS noted that [redacted] entire desk in room [redacted] of Building 1425, was quite dirty. IVINS described [redacted] area as covered with an enormous amount of dust, debris and other material. According to IVINS, other areas in the office, including IVINS' [redacted] desks, were not similarly dirty. Because of the condition of [redacted] desk, and because he knew that [redacted] had worked a great deal with *B.a.*, both in his laboratory and in support of Operation Noble Eagle, IVINS wondered privately whether any of the dust and dirt on [redacted] desk contained *B.a.* spores.[22] According to IVINS' thinking, if a spore was to fall in a dusty area, which had not subsequently been cleaned, the spore could still be present.[23]

Therefore, in December of 2001, IVINS took the independent initiative to swab the office [redacted][24] IVINS specified the following reasons as to why he decided to conduct random environmental sampling within his office around [redacted] desk area: his experience with the powder in the Daschle letter, [redacted] comments of possibly being exposed, [redacted] being administered antibiotics, and information that [redacted] personnel did not utilize safety precautions.[25]

[redacted]

- *IVINS' December 2001 microbiological survey*

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According to IVINS, he personally and privately decided to obtain environmental samplings of [redacted] desk, plate them on Tryptic Soy Agar (TSA), and look to see if any colonies appeared that were consistent with *Bacillus* species. IVINS swabbed approximately 20 areas of [redacted] desk, including the telephone, computer, and desktop. The plates were taped, taken into the B3 suite and incubated overnight. The following day, the plates were examined for colonies that had a colonial appearance consistent with *Bacillus* species. Approximately half of the plates yielded one to two colonies that suggested *Bacillus* species were present.[26] The presumptively positive colonies were not examined by gram stain, nor were they examined for the presence of the plasmids pXO1 and pXO2. IVINS did not examine the colonies on capsule agar.[27]

IVINS autoclaved and disposed of all the samples. IVINS told [redacted] of his findings, but was unsure if he actually showed [redacted] the samples. Because the possibility existed that [redacted] desk contained some *B.a.* spores in the dust and dirt, IVINS decided to thoroughly clean [redacted] desk area, to include everything on the desk.[28] During one interview with the FBI, IVINS stated that he placed the computer keyboard and wrist pad in the pass-thru box to decontaminate them.[29] IVINS did not mention these specific actions during other interviews and in sworn statements. IVINS indicated that he took no remedial measures beyond cleaning up [redacted] desk because he did not consider the level of contamination to be significant. He did not believe that a full decontamination of the room was warranted based on the finding of a few presumptively positive spores. IVINS did not perceive himself or his coworkers to be at risk.[30]

IVINS provided several reasons for swabbing only [redacted] desk area. [redacted] desk contained a layer of dust not present on either IVINS' or [redacted] desk. [redacted] Additionally, the airflow in room [redacted] placed his [redacted] desks upwind from [redacted] desk.[31]

With the exception of [redacted] IVINS told no one at the time of his swabbing efforts and findings. IVINS did not advise [redacted] about the survey of [redacted] workspace, nor did he reveal to [redacted] the finding of presumptive positive colonies.[32] IVINS provided multiple reasons for not reporting his actions.[33] Because he was concerned that records might be obtained under the Freedom of Information Act, IVINS did not keep records, nor did he verify the presence of *B.a.* on the cultures.[34] If the colonies were not *B.a.*, IVINS felt he would have been agitating

[redacted]

many people for no real reason. Additionally, IVINS had no desire to cry "Wolf!" simply because he had found presumptive colonies that were not demonstrated to be *B.a.* or even *Bacillus* species. If the colonies were *B.a.*, he felt that he had effectively taken care of the contamination issue by cleaning [redacted] desk.[35] IVINS reasoned that since USAMRIID personnel were extraordinarily busy at the time processing samples for Operation Noble Eagle, he believed that telling people of a potential breach of containment and that *B.a.* spores were inadvertently taken into non-containment areas would have served no beneficial purpose.[36]

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IVINS acknowledged, in retrospect, that although his concern for biosafety was honest and his desire to refrain from crying "Wolf!" unnecessarily was sincere, he should have notified his supervisor ahead of time of his worries about a possible breach in biocontainment. IVINS thought that quietly and diligently cleaning the dirty desk area would both eliminate any possible *B.a.* contamination, as well as prevent unintended anxiety and alarm at USAMRIID.[37] IVINS indicated that he did not seek permission to conduct the survey, and he did not voice his concerns about a possible breach in containment because he felt that [redacted] at the time, would have been dismissive of [redacted] concerns.[38]

[redacted] confirmed that IVINS had informed [redacted] two days after he conducted an environmental survey of [redacted] desk area in December 2001.[39] IVINS told [redacted] that he had swabbed [redacted] office because he was concerned about the comments made by [redacted] IVINS advised [redacted] that *B.a.* appeared to be present in some of the samples. During an FBI interview, [redacted] indicated that [redacted] was not concerned by IVINS' news because it could have been anything, and [redacted] was vaccinated.[40]

[WRITER'S COMMENTS - IVINS' justifications of his actions following the independent sampling contradicted his explanation of motives for conducting the survey in the first place. If truly motivated by a concern of contamination on the cold-side of USAMRIID, upon evidence of such contamination, why did IVINS not pursue more extensive methods toward correcting the problem? How would improper handling of B.a. samples be corrected without passing along information that the current containment system was not working properly? Why even conduct the survey in the first place if he did not want to cause alarm, especially if he suspected that he would find contamination? IVINS had the courage to conduct the swabbing without command approval, yet lacked the initiative to inform the appropriate authorities when the results were presumptively positive? IVINS was obviously concerned enough about possible contamination to knowingly

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violate USAMRIID protocol, yet at the moment his concerns were validated, he took no actions toward addressing the problems for the benefit of USAMRIID.

If motivated by a concern for [redacted] why did IVINS not inform [redacted] of the presumptive positive results from [redacted] desk? IVINS confirmed during an interview that he did not inform [redacted] of his findings; however, reasons for not telling [redacted] were not provided.[41] The assessment by IVINS that the level of contamination within the office was not a health risk to himself, [redacted] did not diminish the significance of finding contamination outside of the hot suites. b6 b7C

IVINS gave conflicting accounts of exactly when he informed [redacted] about the environmental survey. In IVINS' AR 15-6 sworn statement, he indicated that he mentioned to [redacted] that he was going to check [redacted] desk in December and told [redacted] after he had done so that about half of the cultures were suspicious for anthrax. During other interviews, IVINS stated that he told [redacted] of the swabbing after the fact.

In addition to expressing minimal uneasiness with regard to [redacted] safety concerns, [redacted] presented a cavalier attitude with respect to the results of IVINS' December swabbing results.]

SPILL INCIDENT IN SUITE B3 ON APRIL 8, 2002

- *Description of April 8, 2002, incident in suite [redacted]*
  On April 8, 2002, [redacted] removed eight 2-liter flasks containing *B.a.* spores from a shaker incubator in room [redacted] of suite B3. The flasks, containing erythromycin-resistant (erm+) *B.a.*, were transported in two trips on a cart to [redacted] laboratory in room [redacted] of suite B3. [redacted] put the flasks in the hood and noticed that the paper towel taped over the cap of one of the flasks was discolored on the inside. [redacted] also noticed dried media on the sides of several of the flasks. [redacted] notified everyone in the suite of the potential exposure and bleached off the flasks. In addition, [redacted] notified [redacted] within minutes. [redacted] were sent by [redacted] to the ward for nasal swabs and evaluation.[42] b6 b7C b2 b7F

- *Results of [redacted] suite B3 environmental survey*
  [redacted] instructed [redacted] to perform surveillance cultures of multiple rooms inside the B3 suite on April 9-11, 2002, to determine the extent of contamination resulting from the spill.[43] Surface contamination by *B.a.* was identified in several b6 b7C

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areas of the suite. Some of the positive cultures in the B3 suite were emr+, while others tested erm-. The erm-resistant (erm+) strain contamination followed [redacted] tracks in the laboratory suite.[44]

Approximately 31 colonies of *B.a.* were cultured from the handle of the B3 pass-box inside the suite.[45] The colonies derived from the pass-box handle were not tested for erythromycin sensitivity because [redacted] did not access the pass-box on the day of the laboratory accident. An erm- result was obtained for a colony from the B3 break room where the pass-box was located, indicating that the contaminating colony was not associated with the April 8, 2002, accident.[46] [redacted] was concerned that [redacted] found contamination on the latch of the pass-thru box, as the area was the point of entry and exit from materials into and out of B3.[47] All areas with contamination were cleaned with 20% bleach and were negative upon resurvey by swab culture.

[redacted] indicated that the level of contamination found during [redacted] survey of suite B3 was approximately the same as present on many occasions when routine surveillance was performed in the hot suite.[48] [redacted] advised that [redacted] findings did not appear out of the ordinary for a laboratory environment. Though spores were located on the "hot-side" handle of the pass-thru box, sampling inside the box was negative, which indicated to [redacted] that a breach in containment from the "hot-side" to the "cold-side" had not occurred.[49]

Following the suite-wide sampling, [redacted] held a meeting with the [redacted] Division to advise of [redacted] findings and to notify personnel that "periodic sterility checks" would be re-instituted immediately.[50] According to [redacted] prior to the April 8, 2002 incident, bacteriological monitoring of the BSL-3 was not conducted routinely.[51] Until a number of years prior to 2002, weekly surveillance was performed. [redacted] believed that the practice stopped when anthrax research diminished and had not been reinitiated on a regular basis.[52]

Contrary to [redacted] IVINS felt that the total intensity of contamination from [redacted] suite-wide survey was greater than expected and also of special concern, since spores were found on the inside pass-box latch, as well as on shoes and articles of clothing in the hot-side change rooms.[53] On Monday, April 15, 2002, IVINS suggested to [redacted] that they also swab parts of the cold-side for possible contamination. IVINS suggested areas to sample, including his office, the cold-side of the pass-thru box, and shower shoes in the cold-side men's change room. [redacted] did not understand why IVINS made this suggestion

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because there was no indication of a breach in containment from the hot-side to the cold-side. IVINS indicated that he was concerned about the pass-thru box because that was where *B.a.* entered into B3. In addition, he was concerned about an incident relayed to him by [redacted] in approximately November 2001. IVINS informed [redacted] that in November 2001, [redacted] saw a USAMRIID scientist wearing a glove that had been in the hot suite and carrying the Daschle letter sealed in a plastic bag. IVINS told [redacted] that he and [redacted] considered the incident to be unsafe practice, and that he was worried about contamination issues.[54] IVINS also informed [redacted] that in December 2001, due to the aforementioned glove incident, he swabbed [redacted]' desk in the office shared by IVINS, [redacted] He read presumptive positives on a couple of plates, but did not do any further testing to definitively determine if the spores were *B.a.*[55] [redacted] told IVINS that if he never confirmed the spores were *B.a.*, then the issue was not worth talking about. [redacted] discounted the importance of IVINS' results since he did not consider the issue important enough to confirm the results.[56] [redacted] felt that IVINS' concerns were an overreaction to a relatively minor incident. [redacted] specifically told IVINS not to conduct any sampling without first obtaining approval from [redacted]

According to [redacted] advised [redacted] on April 15, 2002, that IVINS had approached [redacted] about conducting a sampling survey on the B3 pass-thru box on the cold side. [redacted] strongly advised IVINS against conducting any sampling. [redacted] did not think that IVINS would conduct unapproved sampling.[58]

[WRITER'S COMMENTS - Interesting to note that in all of his interviews and statements regarding the independent survey, IVINS never mentioned conversing with [redacted], nor did IVINS relay [redacted] strong advisement against unauthorized sampling. IVINS made no mention to [redacted] of his concerns regarding the analysis of the Daschle letter and powder in suite B3, or his perception of unsafe handling of B.a. evidence by [redacted] Despite being advised against such unauthorized action, IVINS went forward with his independent sampling mission.]

IVINS' APRIL 2002 INDEPENDENT ENVIRONMENTAL SURVEYS

- *IVINS' motivations for conducting additional unauthorized environmental surveys*

On April 11 or 12, 2002, IVINS became aware of the contamination incident in suite B3. In addition, he learned that [redacted] had conducted environmental sampling in the suite and

[redacted]

found several areas with presumptive positives for the presence of *B.a.*[59] On the following Monday, April 15, 2002, after thinking over the weekend and becoming increasingly more concerned about possible contamination immediately outside the [redacted] suite, IVINS decided to independently conduct environmental surveys in areas likely, in his mind, to have been contaminated. IVINS acknowledged that he should have requested authority from his superiors to conduct the surveys, but he was afraid that it would have taken weeks before permission was granted due to "red-tape."[60] IVINS felt that he had a legitimate concern due to the possibility of the area being contaminated.[61]

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- *IVINS' April 2002 microbiological survey*

Without approval or consultation with anyone in the chain of command, IVINS swabbed a number of locations outside the [redacted] biocontainment suite on April 15-16, 2002.[62] On April 15, 2002, IVINS surveyed the cold-side of the [redacted] pass-thru box and the cold-side men's changing room [redacted] of suite [redacted] In addition, he again swabbed [redacted] desk area. According to IVINS, he sampled the cold-side of the pass-thru box because [redacted] survey yielded a presumptive positive for *B.a.* on the handle of the pass-thru box inside the [redacted] suite. IVINS swabbed the cold-side men's changing room because he was concerned about shoes worn inside the [redacted] suite tracking spores into the changing room. IVINS re-swabbed [redacted] desk area because he was concerned that since [redacted] was still working in and around the *B.a.* evidence, [redacted] may have inadvertently contaminated the area. He also wanted to check whether he had done a good job cleaning the desk in December 2001.[63]

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On April 16, 2002, IVINS analyzed the results of the previous day's survey and found presumptive positives for *B.a.* on five of 25 samples. The positives were found on: 1) the molding by the pass-thru box, 2) the men's changing room table, 3) the shelf over the sink in the men's changing room, 4) on top of the lockers in the men's changing room, and 5) on top of [redacted] computer hard drive.[64] Based on the findings, IVINS continued his independently initiated swabbing survey by obtaining samples from additional areas, to include his office desk area located in room [redacted], the cold-side [redacted] pass-thru box area, and the freezers located in the hallway outside the [redacted] suite. The areas on which IVINS concentrated were those areas of high traffic, such as a computer keyboard, and those areas of very low traffic, such as the top of a book shelf. Of the 31 samples collected on the second day, IVINS identified approximately 11 which appeared to be presumptive positives. The initial positives from the April 16, 2002, survey were found at the following locations: 1) outside the pass-thru box on the window sill, 2) outside the pass-thru box on the electrical box, 3) IVINS' office on the

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shelf under the air vent, 4) IVINS' office behind the computer monitor, 5) IVINS' office on IVINS' desk by the penholder, 6) IVINS' desk on the penholder, 7) IVINS' office on the upper shelf in a tray to the left corner air vent, 8) IVINS' office on the metal folder holder on the top shelf to the right of corner air vent, 9) IVINS' office, top shelf of desk, left corner air vent, 10) IVINS' desk, and 11) IVINS' office on the wooden bookcase shelves to the left of the desk.[65] Of the 11 presumptive positives, polymerase chain reaction (PCR) analysis later eliminated numbers 1 and 6, indicating that the bacterial colonies were not *B.a.*[66]

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[WRITER'S COMMENTS - IVINS' explanations of his motivations for the April 2002 independent survey were contradictory to his actions following the December 2001 survey. If IVINS continued to be legitimately concerned that [redacted] was contaminating the office space, why did he not inform [redacted] of his previous swabbing results, or give [redacted] some guidance with regard to safe handling of B.a.? One of IVINS' previous arguments for not notifying USAMRIID command of his December 2001 swabbing was that he believed that he had sufficiently cleaned the contaminated desk area; however, IVINS used the possibility that [redacted] desk was not completely decontaminated in December 2001 as one of the reasons to justify further swabbing. [redacted] survey of the [redacted] suite yielded no indication of a breach in containment from the hot-side to the cold-side. Why were IVINS' convictions so strong regarding possible contamination on the cold-side, while the concerns of other experienced researchers, such as [redacted] and [redacted] were satisfied by the results of [redacted] survey within [redacted]

- *Results of IVINS' April 2002 environmental survey*

In total, 56 samples were plated by IVINS on April 15-16, 2002. Of the 25 sites surveyed for the presence of *B.a.* on April 15, five presumptively positive samples were plated onto capsule agar, and all five produced mucoid growth after incubation. Of the additional 31 sites surveyed on April 16, 11 presumptive positives were obtained. None of the 11 presumptively positive colonies were plated on capsule agar.[67] Colony counts from all but one swipe indicated a very low level of contamination, approximately one to three spores. A single swipe from just outside the [redacted] pass-box yielded greater than 200 spores. On April 18, 2002, 12 suspicious colonies, isolated from IVINS' April 15-16, 2002, cultures, were provided to USAMRIID's [redacted] for *B.a.* confirmation via PCR.[68]

- *Responses to IVINS' April 2002 survey*

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On April 16, 2002, after viewing the culture results from the first day of sampling, IVINS informed [redacted] that he

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had found presumptive positives for the presence of *B.a.* outside the [redacted] suite. IVINS told [redacted] that he intended to conduct further sampling in their office and asked if he could sample [redacted] desk area. [redacted] declined the offer to have [redacted] area sampled.[69] [redacted] stated that [redacted] did not become involved in the issue because [redacted] had other things to do.[70]

According to [redacted] on April 16, 2002, [redacted] IVINS stating that IVINS needed to talk to [redacted] Eventually, IVINS informed [redacted] that he found presumptive positives for *B.a.* outside of the [redacted] suite.[71] [redacted] indicated that he had no prior knowledge of the surveillance cultures of several locations outside the suite.[72] According to [redacted] who viewed the cultures, one appeared to be a "pure colony" of about 200 spores, which was not considered a significant amount. The other samples yielded one to two colonies and appeared "dirty" with other organisms.[73] According to [redacted] on April 16, 2002, [redacted] was advised by [redacted] that IVINS had identified contamination outside the [redacted] laboratory. [redacted] reported this information to [redacted] [redacted] advised [redacted] that IVINS told [redacted] about the swabbing after the fact.[74] [redacted] was upset that IVINS conducted the sampling without going through the appropriate channels for approval.[75]

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On either the afternoon of April 17, 2002, or the morning of April 18, 2002, IVINS told [redacted] about his sampling survey and the findings. [redacted] became very upset with IVINS and told him that he should have gotten approval.[76] According to IVINS, on April 18, 2002, he went to [redacted] and told [redacted] of the sampling survey and the findings. [redacted] was also upset with IVINS for sampling without authority.[77] Likewise, USAMRIID's command staff was very upset with IVINS' actions.[78] According to IVINS, [redacted] angry reaction to learning of IVINS' independent environmental sampling confirmed IVINS' suspicions that [redacted] would have opposed the survey if IVINS had sought preapproval.[79]

According to [redacted] on April 17, 2002, during a conversation between [redacted] and IVINS, IVINS stated that it would be interesting to see what was lying around the facility. [redacted] took the statement to mean that IVINS was curious as to what microorganisms would be found if a sampling survey was conducted.[80]

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[WRITER'S COMMENTS - During an interview on March 31, 2005, IVINS claimed that the path he chose to swab was the path that the Daschle letter took from [redacted] to that through the pass-box in the wall of suite B3.[81] To the contrary, IVINS did not swab the hallway or locations near [redacted] Aside from the areas

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near the B3 pass-box and the freezers in the hallway outside the B3 suite, IVINS did not extensively survey the hallway leading to B3. Of the 56 samples collected on April 15-16, 2002, 38 samples were obtained from the shared office space in room [redacted] 8 samples were collected from the men's locker room, and 10 samples derived from locations near the B3 pass-box and the tops of freezers in the hallway between the B2/B3 hallway.[82] *Based on IVINS' claim that he swabbed the path of the Daschle letter, and given the fact that over half of the survey samples derived from his office, the following question could be posed: Did IVINS have reason to suspect contamination in his office because he had intimate knowledge that the Daschle letter was present in room [redacted] at some point in time?*

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IVINS expressed, during multiple interviews and in sworn statements, his concerns regarding the unsafe laboratory practices of DSD employees. As a justification for his unauthorized environmental surveys, IVINS cited information that DSD personnel did not utilize safety precautions. However, during neither the December 2001 survey, nor the April 2002 survey, did IVINS swab areas associated with DSD laboratories or personnel, aside from [redacted] desk. According to IVINS, [redacted] inwardly seemed pleased with IVINS' finding of contamination because it allowed [redacted] to "point a finger" at DSD for poor laboratory safety procedures.[83] *Notably, the majority of the B.a. contamination identified on the cold-side of Building 1425 was in locations associated with IVINS more so than DSD.*

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[redacted] [redacted] informed [redacted] of the results of IVINS' independent environmental survey. [redacted] regarding IVINS' survey [redacted]

AFTERMATH OF IVINS' APRIL 2002 INDEPENDENT SURVEYS

On April 16, 2002, in response to IVINS' independent survey, [redacted] directed [redacted] to collect samples from around the cold-side of the pass-thru box, where IVINS located spores.[84] [redacted] went to the pass-thru and noticed mortar dust on the ledge by the box. [redacted] assumed that the dust derived from drilling above the area during renovation work. [redacted] took a sample of the dust, which later yielded negative results for *B.a.*

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contamination. Approximately thirty minutes to an hour after the initial collection, [redacted] returned to the pass-thru box to conduct further sampling. [redacted] then learned from IVINS that he had wiped down the area around the pass-box with water. IVINS stated that he did not use bleach because he did not want to panic anyone with the strong bleach odor.[85] In total, [redacted] collected 16 samples on April 16, 2002, from areas near the B3 pass-box and from the women's B3 change room.

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On April 18, 2002, [redacted] was instructed to survey additional sites to obtain a preliminary assessment of the extent of contamination away from the pass-box. An additional 51 swipes were taken in the common areas around suites B2 and B3, and in Bacteriology offices located in rooms [redacted][86] A second surface decontamination was performed in the corridor where the B3 pass-box was located. Locker surfaces in the B3 clean-side men's change room and desk surfaces in room [redacted] were also decontaminated.[87] All of the plates derived from [redacted] April 16 and 18, 2002, surveys possessed growth of mixed environmental microbial flora; however, all of the cultures were negative for *B.a.*-like colonies.[88]

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On the morning of April 19, 2002, a "town hall meeting" was conducted at USAMRIID to discuss the incident which occurred in the B3 suite regarding the spill by [redacted] and the ensuing environmental surveys. Personnel were notified that a large scale sampling survey would be conducted to determine the extent of contamination outside the containment laboratories.[89]

Shortly before the meeting, [redacted] discussed IVINS' environmental survey and the results [redacted] the discussion [redacted] was the first time [redacted] learned that spores were found on the computer keyboard in [redacted] office. [redacted] was very upset that IVINS swabbed the office and did not tell [redacted] More importantly, [redacted] upset that IVINS had found spores and did not tell [redacted] [redacted] did not think that IVINS thought through his act of swabbing areas of the cold side. [redacted] knew of no independent swabbing by IVINS prior to April 2002.[90]

[WRITER'S COMMENTS - As of April 25, 2002, [redacted] It is unclear if, or when, [redacted] was ever notified of the December 2001 survey results.]

- *USAMRIID-wide microbiological survey*

On April 19, 2002, an extensive environmental survey of USAMRIID's Building 1425 was conducted. Samples were collected from areas throughout the building, including non-BSL-3 laboratories, office areas, corridors, animal areas, maintenance

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areas, support areas and pass-boxes outside all laboratories. Samples were acquired from the Building 1425 break room and 22 offices with the following room numbers: [redacted] and [redacted] On April 19, 2002, a total of 838 samples were collected from 86 sites within the building. A total of 107 non-hemolytic, bacillus-like (NHB) colonies were obtained from the 838 samples.[91]

On April 20-21, 2002, personnel from the Center for Health Promotion and Preventative Medicine (CHPPM) of Aberdeen Proving Ground, Maryland, conducted additional sampling. CHPPM Survey Set #1 included the laundry center at the Jeanne Bussard Center and the DIS equipment. A total of 58 samples were collected during CHPPM Survey #1 on April 20, 2002.[92] Five NHB colonies were obtained from five sites. On April 21, 2002, CHPPM Survey Set #2 included sampling in the following areas: B1, the B2/B3 hallway, the pass-box to [redacted] the men's clean change room [redacted], and room [redacted] A total of 113 samples were collected from nine sites within the building during CHPPM Survey #2. Of the 113 samples, nine NHB colonies were obtained.[93]

On April 23-25, 2002, supplemental surveys of the Budget office, room [redacted] and room [redacted] were conducted. Of the 65 total samples collected, two sites yielded a total of two NHB colonies.[94]

- *Results of USAMRIID-wide microbiological survey*

Of the 1074 samples collected during the environmental surveys on April 19-25, 2002, a total of 123 NHB colonies were isolated from 102 sites. The 123 NHB colonies were identified as Bact #1 through Bact #123.[95] The NHB colonies were subjected to determination of sensitivity to gamma phage and to PCR analysis for the presence of genes for capsule and protective antigen (PA). Of the 123 total NHB colonies, only two colonies were confirmed positive for the presence of both *B.a.* capsule and PA genes. Notably, the two positives were obtained in locations where positive results were previously reported following IVINS' independent survey. The two colonies, identified as Bact #115 and #116, were confirmed positive for virulent *B.a.* and were later genotyped as Ames. Bact #115 was obtained during CHPPM Survey Set #2 and was described as "C-99, clean-side change room, [redacted] locker top." Bact #116 was obtained during CHPPM Survey Set #2 and was described as "C-78, rm. [redacted] bookcase, rt, inside door."

On May 8, 2002, the genotypes of 27 *B.a.* colonies derived from the surveys were reported. Two of the colonies resulted from the USAMRIID-wide microbiological survey, while the

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remaining 25 colonies derived from IVINS' cultures of April 15-16, 2002. Of the 27 colonies, 20 were typed as the Ames strain, four were typed as the Vollum 1B strain and two were typed as the Sterne strain. No product was obtained from the analysis of one of the 27 colonies. The 27 colonies represented *B.a.* contamination in only three locations. Of the 15 colonies obtained from room [redacted] (IVINS' office), 10 were Ames strain, two were Sterne strain, two were Vollum 1B, and one yielded no product during PCR analysis. Of the six colonies derived from the cold-side of the B3 pass-box, all six typed as Ames. Of the six tested from the men's B3 change room, four were *B.a.* Ames, and two were *B.a.* Vollum 1B.[96]

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[WRITER'S COMMENTS - Following is a summary of the environmental survey results, incorporating IVINS' data with that obtained in the follow-up surveys. Between April 15-25, 2002, approximately 1197 samples were collected from over 100 locations. Of the 1197 samples, 16 cultures tested positive for the presence of virulent B.a. Two of the B.a. cultures derived from the USAMRIID-wide survey, while the remaining 14 derived from IVINS' surveys on April 15-16, 2002. IVINS plated a total of 51 samples, 27% of which yielded virulent B.a. colonies. Only two of the 1141 samples, or 0.18%, derived from the surveys conducted in response to IVINS' independent swabbing, yielded virulent B.a. colonies. Of the over 100 sites surveyed in Building 1425, positive results for the presence of virulent B.a. were obtained in only three locations: the men's change room in [redacted] the cold-side B3 pass-box, and room [redacted] Notably, these were the three sites originally surveyed by IVINS. Of the 22 offices surveys, only the office shared by IVINS, [redacted] and [redacted] tested positive for the presence of virulent B.a.]

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- *Comparison between IVINS' survey and the USAMRIID-wide survey*

When asked why he was able to find virulent *B.a.* when the other swabbing efforts did not, IVINS explained that he utilized a more aggressive and extensive method for collecting samples. According to IVINS, he sampled a larger area, especially hard to reach and dusty areas, and used more force. He also explained that prior to the large scale swabbing survey by CHPPM, [redacted] took samples outside the [redacted] laboratory and subsequently cleaned the area thoroughly with bleach.[97] IVINS stated that he believed there were additional areas that were contaminated; however, everyone had cleaned their areas as a preventative measure. IVINS stated that the random sampling conducted by USAMRIID safety staff consisted of about 6-10 swabbings in high traffic areas, such as the phone, desk, and computer. He felt the technique was inadequate. IVINS asserted that he took

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approximately 80 swabbings from his office alone, from high traffic areas, and other areas near ventilation ducts.[98]

SPECULATIONS REGARDING IVINS MOTIVATIONS FOR CONDUCTING INDEPENDENT SURVEYS

[redacted] who was the [redacted] in April 2002, discussed two possible reasons why IVINS conducted microbiological contamination surveys in the non-containment areas without command staff approval: (1) IVINS did not follow appropriate standard operating procedures (SOPs) while working with material and thought he may have contaminated some clean areas of USAMRIID, or (2) IVINS noticed another individual not following SOPs and wanted to bring the problem to light. [redacted] did not believe that IVINS was covering up a *B.a.* contamination trail which may be detected by the survey, nor did [redacted] believe that IVINS was involved in any criminal behavior relating to the AMERITHRAX investigation.[99]

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[redacted] suspected that IVINS decided to conduct random sampling of cold areas because of the contamination incident concerning [redacted] [redacted] stated that the finding of contamination in the pass-box area within the laboratory prompted IVINS to test the exterior of the pass-box, as well. With regard to [redacted] office in room [redacted] [redacted] suspected that IVINS conducted sample testing due to the possibility that [redacted] became contaminated while working in [redacted] According to [redacted] IVINS had heard a rumor that the people in [redacted] did not follow safety precautions. IVINS feared that [redacted] may have cross-contaminated [redacted] due to lack of proper procedure by people from [redacted][100]

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[redacted] did not know why IVINS conducted random contamination sampling of the cold areas, but indicated that he may have gotten word of the glove incident with [redacted][101]

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[WRITER'S COMMENTS - During an interview with the FBI, IVINS noted a long-standing political rivalry between the Bacteriology Division and DSD at USAMRIID. IVINS stated that Bacteriology Division employees felt ostracized and belittled by DSD researchers who were reportedly loathe to consult Bacteriology employees' superior expertise.[102] One could argue that IVINS was partially motivated to conducting the independent survey by the desire to make DSD look incompetent with regard to the safe handling of B.a.; however, such an argument is weakened by the fact that, aside from [redacted] desk, IVINS did not survey any locations directly associated with DSD.]

SPECULATIONS REGARDING THE SOURCE OF CONTAMINATION

The Findings of the AR 15-6 Investigation reported that a single source of contamination was not unambiguously identified. Contamination was attributed to inadequate decontamination of the outside of shipping containers brought into and out of suite B3 of Building 1425. Additionally, it was speculated that opening of evidentiary material in suite B3 created conditions leading to contamination outside of ziplocks used to transport material out of the B3 pass-box and led to contamination of the pass-box areas.[103]

According to [redacted] That strain was different from the strain causing contamination of the USAMRIID cold areas. The latter strain was non-erythromycin resistant. [redacted] concluded that the contamination was caused by two different sources of *B.a.*[104] A relationship was not established between the surface contamination within the biocontainment suite and that detected on the outside adjacent areas.[105] [redacted] advised that there was no way of determining how long the cold-side areas had been contaminated, as no prior efforts were conducted by USAMRIID to identify possibly contaminated areas.[106]

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IVINS suspected that poor housekeeping and lack of environmental controls caused the contamination. He further speculated that the contamination resulted from USAMRIID undertaking the role of examining evidence from law enforcement agencies, while lacking a policy or plan in effect to complete the mission. According to IVINS, USAMRIID had never dealt with *B.a.* affixed to a powder and the anthrax letter evidence may have arrived at USAMRIID in an outer package contaminated with *B.a.*[107] Specifically, IVINS speculated that *B.a.* detected on the cold-side Building [redacted] may have been contamination from the Daschle letter.[108]

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With regard to the contamination found on his desk, IVINS indicated that he would sometimes set containers in ice buckets on his desk so that he would remember to ship the material or to fill out paperwork. The containers would remain on his desk for a few hours or sometimes overnight. When live *B.a.* was received from outside of USAMRIID, IVINS never opened the outer shipping container in the office. When IVINS took *B.a.* to Building [redacted] he would either take it over directly or store it in the refrigerator just outside of [redacted] until he was ready to go to Building [redacted] Typically, he would not stop by his desk.[109]

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[redacted] advised that [redacted] did not know how [redacted] office, or the other areas, became contaminated with *B.a.* spores. [redacted] suggested the possibility that DSD may have received an item that was not containerized properly and the container exterior was contaminated.[110] [redacted] also speculated that the contamination outside of the BSL-3 came from the anthrax letters.[111] Due to the fineness of the powder that USAMRIID personnel tested and came into contact with, [redacted] thought it plausible that individuals may have inadvertently transported the spores from one laboratory to another and to cold or administrative areas. As an example, [redacted] recounted the glove incident [redacted] which [redacted] suspected was against protocol.[112]

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[redacted] speculated that the contamination outside the BSL-3 derived from outside of the laboratory. [redacted] based [redacted] response on the pattern of contamination near the pass-box. [redacted] noted that material was frequently returned from Building 1412 after aerosol challenges. [redacted] also referenced incoming samples from USAMRIID's [redacted] Dugway, Bioport, and other outside agencies.[113]

[redacted] offered no independent ideas as to where the contamination outside of the BSL-3 came from, but indicated that [redacted] heard other people's theory that contamination may have come from the Daschle letter.[114] [redacted] recalled that on one occasion a letter that was identified as contaminated with anthrax was taken into suite B3 by IVINS, and that the letter could have been the source of contamination within the cold areas of USAMRIID.[115]

[WRITER'S COMMENTS - If the contamination derived in part from the handling of evidentiary material, to include the Daschle letter, then why was contamination not also identified in [redacted] areas, where the bulk of the evidentiary analyses were conducted and where all incoming unknown items were received?]

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NOTABLE REFERENCES BY IVINS TO HIS INDEPENDENT SURVEYS

IVINS referenced his independent surveys and the perceived backlash in multiple email messages to [redacted] On June 16, 2002, at 9:18 p.m., IVINS sent a message to [redacted] stating "the atmosphere around USAMRIID is so poisonous these days that it's hard to get much of anything done. I'm still persona non grata, especially with people in power, like [redacted] seems to bark at me about lots of things these days." On July 7, 2002, at 9:04 p.m., IVINS sent [redacted] a message indicating that [redacted]..Spring is when I found the break in

[redacted]

mutant B. anthracis onto guinea pigs in the vet med cold suites. The pigs started dying. I cultured their blood and did a plasmid screen and found they had died from fully virulent pX01+, PX02+ B. anthracis. Animal caretakers had taken dead pigs and put them into the cold room. There was a lot of local decontamination in rooms, hallway, coldroom, but nothing was mentioned outside of this place. I am now forbidden from being a "cowboy." I can't think for myself, and I can't do anything without everybody up and down the line questioning me about it. I'm sure it's punishment..."

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During an interview on March 31, 2005, IVINS indicated that he had no concerns about [redacted] competency as a laboratory worker, and his decision to survey [redacted] work area was not influenced by any perception that [redacted] was negligent in [redacted] safety practices.[117] However, IVINS send [redacted] multiple email messages expressing views regarding [redacted] performance in the laboratory which were quite contrary to his interview statements. On August 20, 2001, IVINS commented extensively on [redacted] performance in the laboratory. [redacted] to make some Sterne spores last week, and to have them ready for today. [redacted] didn't have any backup blood plates, and [redacted] didn't inoculate the cultures until Friday. Today when [redacted] the Leighton and Doi cultures were a total bust, no spores inside or outside the cells. [redacted] made some L&D medium [redacted] and inoculated some Ames culture flasks, and they made spores fine. [redacted] and I think that maybe [redacted] messed up the salts, [redacted] been having a lot of problems lately making good spores. It seems [redacted] rushes through the work so that [redacted] It's very discouraging when I know that [redacted] doesn't even take the slightest of [redacted] On July 6, 2001, IVINS wrote "more [redacted] problems... [redacted] had not vortexed the spore preps enough to get out the clumps, and so had very erratic counts. [redacted] did a heat shock with no water in the beaker holding the tubes and hardly any water (about 1/8 inch) in the water bath...If I say anything about ANYTHING to [redacted] On April 29, 2001, IVINS wrote [redacted] had problems with simple mathematical calculations, [redacted] basically just a satisfactory employee..."

[redacted]

contamination here at RIID and became 'very suspicious' to people. I wouldn't be surprised if they wanted to quiz you about me. Putting together all of the things I've heard about the FBI's 'suspect list,' my guess is that I'm on it (being a middle-aged white male who has made anthrax spores). I can't tell you how unappealing it is to be considered a mass murderer..."

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On June 18, 2002, at 9:00 p.m., IVINS sent [redacted] a message indicating that "the FBI people who talked to me (right after I found the break in containment and got crucified for it) said they thought that my coming back to work after hours was 'extremely suspicious.' I don't think anyone has any idea how peaceful and quiet it can be here after hours. I can come here and just sit, or read, or get on the web. If [redacted] is being bothersome I can go back into B5 or even into B3. (In the evenings, B3 may as well be Mars. I can just sit and think and relax from everything.) Also, it's just a great time to get work done without hassle. It used to be honorable and commendable to work more than 8 hours a day. Now, it's suspicious..."

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During an interview on March 31, 2005, IVINS stated that the December 2001 and April 2002 independent surveys represented the only two times he ever swabbed in the cold areas of USAMRIID outside of the hot suites. According to IVINS, during the early 1990s he conducted swabbing inside the hot suites as part of USAMRIID's routine environmental monitoring program. IVINS conducted the routine sampling at the direction of his supervisors.[116] The information provided by IVINS on March 31, 2005, contradicted information contained in an email message sent from IVINS to [redacted] On July 7, 2002, at 9:04 p.m., IVINS wrote to [redacted] "oh, guess what? This is actually the third time I've found virulent anthrax on the outside of the hot suite. The other two times were hushed up. The first time was in the early 1980s. [redacted] had injected some guinea pigs (and killed them) with the Vollum 1B strain. The used pans of bedding, blood, urine, feces and all, had to be deconned out of the suite, but the autoclaves were not working. So they decided to paraformaldehyde the bedding and ship it out. After the "decon" I - without authorization (bad! bad!) but with plenty of concern - checked the bedding for sterility. I plated some of it out. The results came back after unimmunized cagewash workers had cleaned the pans. The very top of the bedding was sterile, but below the top layer it was quite contaminated, with anthrax and other bacteria. We're just lucky that nobody in cagewash got anthrax. I told people of my finding, and after that, used bedding in pans was never deconned by paraformaldehyde - it just doesn't penetrate. The second time I found - unauthorized! - virulent anthrax outside the suite was when [redacted] supposedly injected avirulent

[redacted]

IVINS also expressed concerns regarding [redacted] propensity for spreading gossip. On August 20, 2001, IVINS sent [redacted] an email message indicating [redacted] has a tendency to exaggerate and to spread nasty gossip, rumors, etc. about people, and I'm going to be right on [redacted] radar for a long time, I fear.. [redacted]

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[WRITER'S COMMENTS: Based on IVINS' written concerns regarding [redacted] propensity to talk, one could speculate that IVINS did not inform [redacted] of his unauthorized surveys due to a lack of confidence in [redacted] ability to keep the information confidential. Notably, however, IVINS did not address either his concerns with [redacted] propensity to spread gossip, or his issues with [redacted] laboratory performance when asked directly about these issues during an interview on March 31, 2005.[118]]

On August 21, 2004, IVINS contacted SSA [redacted] to advise of an article that was published the previous day in the Los Angeles Times. IVINS faxed to SSA [redacted] an article titled "Anthrax Leaks Blamed in Lax Safety Habits: 'Sloppy' investigation of 2002 breaches at a federal biodefense facility," and authored by Time Staff Writer Charles Piller. According to IVINS, the article detailed the results of the environmental surveys conducted at USAMRIID in April 2002. IVINS claimed that the article disclosed information that was confidential to the FBI. IVINS indicated that he was upset to see the information made public. IVINS believed the following statement to be confidential to the FBI: "Three different anthrax strains - two infectious and one a harmless vaccine - were detected outside biosafety labs."[119]

[redacted]

ENDNOTES

1. 279A-WF-222936-302 Serial 1699
2. AR 15-6 Investigation Exhibit #55
3. 279A-WF-222936-302 Serial 1699
4. AR 15-6 Investigation Exhibit #55
5. 279A-WF-222936-302 Serials 1379 and 1700
6. AR 15-6 Investigation Exhibit #19
7. CID Exhibit #7
8. 279A-WF-222936-302 Serial 1379
9. AR 15-6 Investigation Exhibit #20
10. AR 15-6 Investigation Exhibit #43
11. 279A-WF-222936-302 Serial 1699
12. 279A-WF-222936-302 Serial 1700
13. 279A-WF-222936-USAMRIID Serial 1223
14. 279A-WF-222936-302 Serial 1700
15. CID Exhibit #7
16. AR 15-6 Investigation Exhibit #19
17. CID Exhibit #7
18. AR 15-6 Investigation Exhibit #19
19. 279A-WF-222936-USAMRIID Serial 1223
20. 279A-WF-222936-USAMRIID Serial 1223
21. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
22. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
23. 279A-WF-222936-302 Serial 1700
24. 279A-WF-222936-302 Serial 1700
25. CID Exhibit #7
26. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins); 279A-WF-222936-302 Serial 1700, CID Exhibit #7; 279A-WF-222936-USAMRIID Serial 1223
27. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins); 279A-WF-222936-302 Serial 1700; CID Exhibit #7
28. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)

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29. 279A-WF-222936-302 Serial 1700
30. 279A-WF-222936-USAMRIID Serial 1223
31. 279A-WF-222936-USAMRIID Serial 1223
32. 279A-WF-222936-USAMRIID Serial 1223
33. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
34. AR 15-6 Investigation Exhibit #19
35. 279A-WF-222936-302 Serial 1700
36. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
37. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
38. 279A-WF-222936-USAMRIID Serial 1223
39. AR 15-6 Investigation Exhibit #20
40. 279A-WF-222936-302 Serial 1379
41. 279A-WF-222936-USAMRIID Serial 1223
42. AR 15-6 Investigation Exhibit #14 and 17; AR 15-6 Investigation Findings
43. 279A-WF-222936-302 Serial 1701
44. AR 15-6 Investigation Exhibit #18
45. AR 15-6 Investigation Exhibit #17
46. AR 15-6 Investigation Exhibit #14
47. 279A-WF-222936-302 Serial 1701
48. AR 15-6 Investigation Exhibit #17
49. 279A-WF-222936-302 Serial 1567
50. 279A-WF-222936-302 Serial 1567
51. AR 15-6 Investigation Exhibit #12
52. AR 15-6 Investigation Exhibit #17
53. AR 15-6 Investigation Exhibit #19
54. 279A-WF-222936-302 Serial 1567
55. 279A-WF-222936-302 Serial 1567
56. AR 15-6 Investigation Exhibit #17
57. 279A-WF-222936-302 Serial 1567; AR 15-6 Investigation Exhibit #17

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58. 279A-WF-222936-302 Serial 1701
59. 279A-WF-222936-302 Serial 1700
60. 279A-WF-222936-302 Serial 1700
61. CID Exhibit #7
62. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
63. 279A-WF-222936-302 Serial 1700
64. 279A-WF-222936-302 Serial 1700;AR 15-6 Investigation Exhibit #7
65. AR 15-6 Investigation Exhibit #7
66. 279A-WF-222936-302 Serial 1700;AR 15-6 Investigation Exhibit #7
67. AR 15-6 Investigation Exhibit #19 (ExSum - 18 APR 02, Bruce Ivins)
68. AR 15-6 Investigation Exhibit #7
69. 279A-WF-222936-302 Serial 1700
70. 279A-WF-222936-302 Serial 1379
71. 279A-WF-222936-302 Serial 1701
72. AR 15-6 Investigation Exhibit #12
73. 279A-WF-222936-302 Serial 1701
74. 279A-WF-222936-302 Serial 1567
75. 279A-WF-222936-302 Serial 1567
76. 279A-WF-222936-302 Serial 1700; 279A-WF-222936-302 Serial 1567; CID Exhibit #7
77. 279A-WF-222936-302 Serial 1700; CID Exhibit #7
78. 279A-WF-222936-302 Serial 1218
79. 279A-WF-222936-USAMRIID Serial 1223
80. 279A-WF-222936-302 Serial 1699
81. 279A-WF-222936-USAMRIID Serial 1223
82. AR 15-6 Investigation Exhibit #7
83. 279A-WF-222936-USAMRIID Serial 1223
84. AR 15-6 Investigation Exhibits #7 and #21
85. 279A-WF-222936-302 Serial 1567
86. AR 15-6 Investigation Exhibit #23
87. AR 15-6 Investigation Exhibit #7

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88. AR 15-6 Investigation Exhibit #7
89. 279A-WF-222936-302 Serial 1700
90. 279A-WF-222936-302 Serial 1699
91. AR 15-6 Investigation Exhibit #8
92. AR 15-6 Investigation Exhibit #11
93. AR 15-6 Investigation Exhibit #10
94. AR 15-6 Investigation Exhibit #9
95. AR 15-6 Investigation Exhibit #6
96. AR 15-6 Investigation Exhibits #3,4 and 5
97. 279A-WF-222936-302 Serial 1700
98. CID Exhibit #7
99. 279A-WF-222936-302 Serial 1229
100. CID Exhibit #1
101. CID Exhibit #1
102. 279A-WF-222936-USAMRIID Serial 1223
103. AR 15-6 Investigation Findings
104. 279A-WF-222936-302 Serial 1229
105. CID Exhibit #1
106. CID Exhibit #1
107. CID Exhibit #7
108. 279A-WF-222936-302 Serial 3306
109. AR 15-6 Investigation Exhibit #54
110. CID Exhibit #1
111. AR 15-6 Investigation Exhibit #55
112. CID Exhibit #1
113. AR 15-6 Investigation Exhibit #17
114. AR 15-6 Investigation Exhibit #20
115. CID Exhibit #1
116. 279A-WF-222936-USAMRIID Serial 1223
117. 279A-WF-222936-USAMRIID Serial 1223
118. 279A-WF-222936-USAMRIID Serial 1223
119. 279A-WF-222936-USAMRIID Serial 900; 279A-WF-222936 1A 5512

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

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FEDERAL BUREAU OF INVESTIGATION

Date of transcription 06/18/2005

As previously reported, according to United Stated Army Medical Research Institute of Infectious Diseases (USAMRIID) documentation, BRUCE EDWARDS IVINS was granted four hours of annual leave on September 17, 2001. Amerithrax investigators obtained IVINS' time records for the periods ending August 11, 2001 through December 1, 2001. For the two week time period starting September 9, 2001 and ending September 22, 2001, IVINS' time sheet record indicates that he took four hours of annual leave during day two of week two of the pay period ending September 22, 2001. Since the pay period started September 9, 2001, day two of week two of the pay period is September 17, 2001.

Copies of the documents referenced above (the original copies have been previously submitted) have been attached to and made part of this FD-302.

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Investigation on 06/18/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID - 1326 Date dictated

by

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EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		08/11/01	00163

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT
1	2	LY	8								
1	3	LY	8								
1	6	LS	8								
2	2	LS	2								

REG	OT	COMP	HOL	SUN	2ND	3RD	ND	E/H	LV	NP/LV

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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CERTIFICATION: ATTENDANCES AND ABSENCES CERTIFIED CORRECT. OVERTIME APPROVED IN ACCORDANCE WITH EXISTING LAWS AND REGULATIONS FOR NON-EXEMPT FLSA. I DID NOT SUFFER OR PERMIT ANY OVERTIME WORK OTHER THAN AS REPORTED FOR THIS PAY PERIOD.

CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

AUTHORIZED SIGNATURE

8/25/01

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		09/08/01	00154

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			LH 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT
1	3	LA	2								

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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[redacted]

AUTHORIZED SIGNATURE

CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		09/08/01	00154

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			LH 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

[redacted]

AUTHORIZED SIGNATURE

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		09/22/01	00155

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT
2	2	LA	4								

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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[redacted]

AUTHORIZED SIGNATURE

CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		10/06/01	00157

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT
1	5	LA	21								
2	2	LA	21								
2	5	LA	11								

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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[redacted]

AUTHORIZED SIGNATURE

CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		10/20/01	00156

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			LH 0	RG 0	RG 0	RG 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

AUTHORIZED SIGNATURE

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		11/03/01	00156

HRS WORK | 0745 - 1630 | TDC

STD JON | 6EDP M101CA

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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[redacted]

AUTHORIZED SIGNATURE

CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		11/17/01	00155

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	RG 0	RG 0			LH 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

[redacted]
AUTHORIZED SIGNATURE

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EMPLOYEE ID	BLK/GRP	ACT	ORG	EMPLOYEE NAME	PLT ROT	PERIOD ENDING	SEQ NO
280445449	0800	W4GPAA	BBJB	IVINS BRUCE E		12/01/01	00155

STD JON | 6EDP M101CA | HRS WORK | 0745 - 1630 | TDC

	AWS	SUN	MON	TUE	WED	THR	FRI	SAT	SUN	MON	TUE	WED	THR	FRI	SAT
TOUR	0		8|00	8|00	8|00	8|00	8|00			8|00	8|00	8|00	8|00	8|00	
TYP/SFT			RG 0	RG 0	RG 0	LH 0	RG 0			RG 0	RG 0	RG 0	RG 0	RG 0	
GRADED ND															

WEEK	DAY	TYPE HOUR	HOURS	JOB ORDER NUMBER	E/H OTH	LST HR	TEM SFT	NIGHT DIFF	INJ NUM	START TIME	INIT
1	4	LA	8								
1	6	LA	8								

REG		OT		COMP		HOL		SUN		2ND		3RD		ND		E/H		LV		NP/LV	

WK1	IN	OUT	IN	OUT	IN	OUT	WK2	IN	OUT	IN	OUT	IN	OUT
SUN							SUN						
MON							MON						
TUE							TUE						
WED							WED						
THU							THU						
FRI							FRI						
SAT							SAT						

REMARKS:

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CONTAINS INFORMATION SUBJECT TO THE PRIVACY ACT OF 1974 AS AMENDED

[redacted]

AUTHORIZED SIGNATURE

(Rev. 01-31-2003)

FEDERAL BUREAU OF INVESTIGATION

Precedence: ROUTINE

Date: 06/07/2005

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-15-2008 BY 60324 UC BAW/DK/TH

To: Washington Field

From: Washington Field
AMX #3
Contact: [redacted]

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Approved By: [redacted]

Drafted By: [redacted]

Case ID #: 279A-WF-222936-USAMRIID (Pending) - 1327

Title: AMERITHRAX;
MAJOR CASE 184

Synopsis: To summarize the investigation of prescription drugs used by Bruce Edwards Ivins during 2000 and 2001.

Details: Investigation to date has revealed that Bruce Edwards Ivins, Microbiologist, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), was prescribed various medication during 2000 and 2001. Below is a summary of the medication known to have been prescribed to Ivins during 2000 and 2001:

Celexa®, filled 11/16/00 (30 day supply); 12/19/00 (30 day supply); 1/12/01 (30 day supply); 2/06/01 (30 day supply); 05/02/2001 (90 day supply); 06/27/01 (45 day supply) and 7/31/01 (90 day supply), is used to treat depression. Ivins was attending group counseling sessions during 2000 as a result of depression. He appeared to have refilled the Celexa® prescriptions before the supply was supposed to have been complete. Ivins received a 45 day supply of Celexa® on 6/27/01 when he had 33 days remaining on the supply filled 05/02/2001. Additionally, Ivins filled a 90 day supply of Celexa on 7/31/01 when he had 11 days remaining on the 45 day supply filled 6/27/01.

Augmentin®, filled 3/1/01 (7 day supply), is used to treat respiratory infections. [redacted]

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Cephalexin®, filled 10/18/01 (10 day supply), is an antibiotic. On 10/6/01, Ivins cut his finger but did not seek medical attention. Ivins feared the finger was infected and in an electronic email to [redacted] on 10/16/01, Ivins mentions that he should "probably go see someone and start taking antibiotics."

Doxycycline HYC, filled 10/29/01 (10 day supply) is an antibiotic. No additional information has been obtained regarding this prescription.

Diazepam, filled 11/13/01 (6 day supply), is an anti-anxiety drug containing Valium. No additional information has been obtained regarding this prescription.

In an email to [redacted] on 7/30/01, Ivins discussed the fact that he was taking Zyprexa®. Zyprexa® is used on patients diagnosed with Schizophrenia. No additional information has been obtained regarding this prescription.

Ivins acceded to a polygraph examination on 2/28/02 and at that time Ivins advised the polygraph examiner that he was taking Celexa.

An extensive review of electronic mail archives associated with Ivins is ongoing. Additional pertinent information obtained from the review will be documented in a follow-up EC.

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279A-WF-222936-USAMRIID - 1332

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-15-2008 BY 60324 UC BAW/DK/TH

The following investigative steps were conducted by [redacted] and started on 04/05/2005 in Frederick, MD.

BRUCE E. IVINS genealogy was compiled to produce a visual depiction of the family tree for four generations. The source of the information was The Warren County, Ohio Genealogical Society Obituary Records, The National Comprehensive Report on BRUCE E. IVINS, http://www.classmates.com, search results and *Catholic Review Magazine* at www.catholicreview.org search results and email sources.

Dates of Death for C. WILBUR IVINS and his wife MARY R. IVINS, and T. RANDALL IVINS acquired from Warren County Obituaries.

Information regarding the [redacted] was compiled from Warren County Paper obituary section.

Information regarding the brothers of BRUCE E IVINS; [redacted] was procured from email sources.

High school information for BRUCE E. IVINS and [redacted] was acquired from http://www.classmates.com.

[redacted] information procured from email sources and from the [redacted] for BRUCE E. IVINS. As well as, date of birth information relating to BRUCE E IVINS' [redacted]

An internet search of *Catholic Review Magazine* referenced an article mentioning [redacted]

Attached hereto and considered as part of this document are copies of the above mentioned reports.

[redacted]

insertgenealogy0405.wpd

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-15-2008 BY 60324 UC BAW/DK/TH

BRUCE E. IVINS FAMILY TREE

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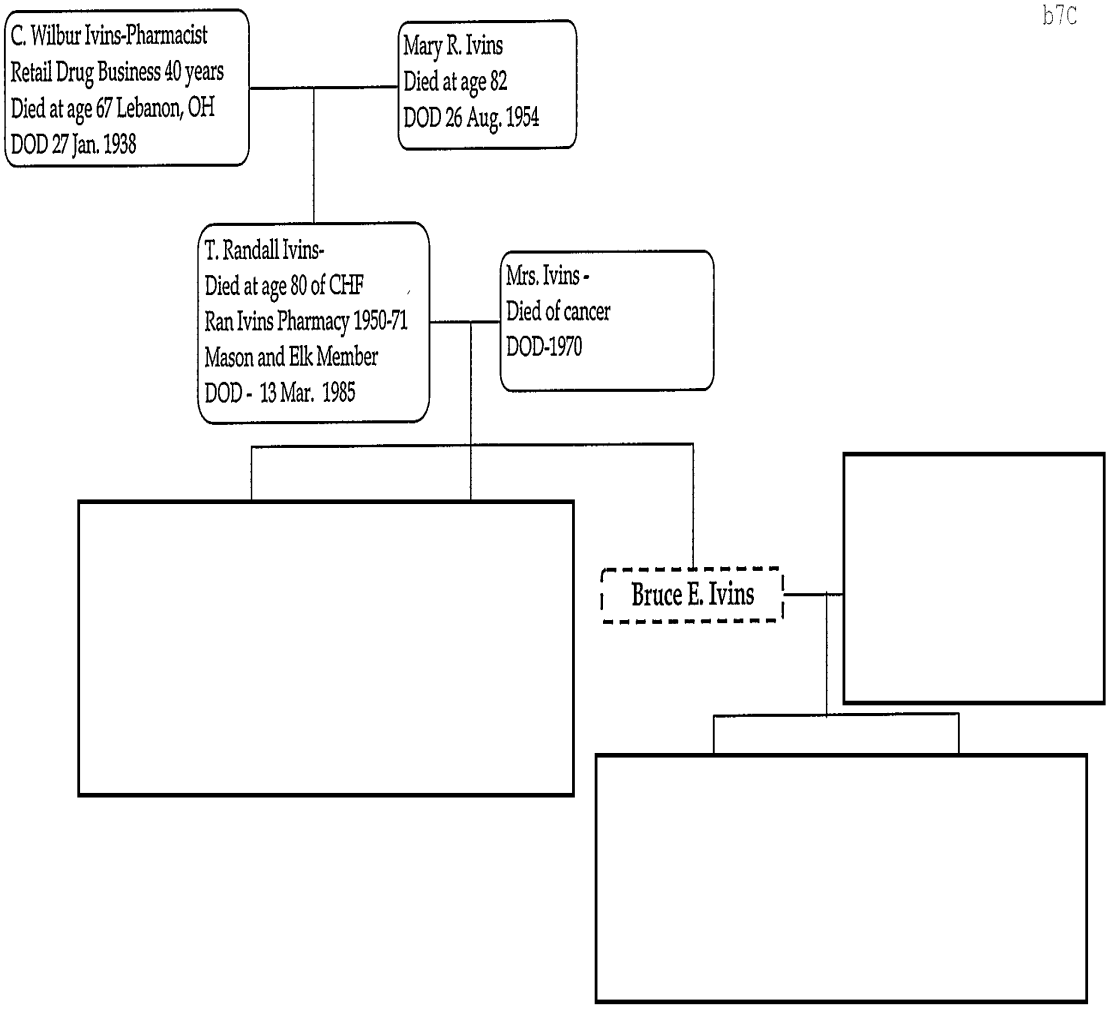

ASIDE: Bruce had his 35th HS Renunion summer of 1999
Lebanon HS 1960-1964

Last Updated 05/2005

Hurwich, Saul
10 June 1954

ILLNESS IS FATAL TO SAUL HURWICH

Associated With Fashion Shoppe Many Years; Rites Tuesday

Following an illness of several months, Saul Hurwich, aged 60, died Sunday at Jewish Hospital in Cincinnati where he had been a patient for several weeks.

He had been associated with his wife Ruby in The Fashion Shoppe here for more than 35 years. A graduate of University of Pittsburgh, he was an attorney and practiced law for a time in Pittsburgh. He was a member of Lebanon Lodge of Masons, Scottish Rite and Shrine in Cincinnati and was past exalted ruler of the Lebanon Lodge of Elks.

Funeral services were held at 2 p. m. Tuesday at Weil Funeral Home in Cincinnati with burial in Walnut Hills Jewish Cemetery.

Besides his wife he leaves one brother and four sisters.

Hutchinson, Otto
10 June 1954

Otto Hutchinson Succumbs At 78

Funeral services for Otto Hutchinson, aged 78, of Clarksville, Route 1, retired farmer who died at Miars Rest Home Sunday were held at 10:30 a. m. Wednesday at Vale Funeral Home in Morrow with burial in Maineville Cemetery. He had been in ill health for eight years.

Surviving are his wife, Tressie, two sons, Arthur of Clarksville Route 1 John of Blanchester Route 2, six grandchildren and one great grandchild.

Hutchinson, Russell
11 Feb 1954

Brother Of Local Resident Called

Russell A. Hutchinson of Toledo, brother of Mrs. Ralph Lewis, was in a serious condition and on his way to Phoenix, Arizona. Mr. Lewis accompanied his brother-in-law but Mr. Hutchinson couldn't continue his fight for life and in New Mexico passed away on Saturday, February 6, at 10 p.m. Mr. Lewis is accompanying the body home, arriving Wednesday. No funeral arrangements have been made.

Hutslar, Esther
27 May 1954

LOUISIANA WOMAN IS COUNTY'S SIXTH TRAFFIC VICTIM

Killed Beneath Own Car In Mason Wednesday Night

celerator. The car knocked Mrs.

death of the year was recorded Wednesday night when a 56-year-old New Orleans woman was killed underneath her own car in the village of Mason.

Police Chief Elmer Wright, who investigated, reported Mrs. Esther Hutslar was en route to Springfield to visit a nephew. She became tired of driving and asked her companion, Mrs. Gertrude Haase, 52, New Orleans to relieve her. They stopped near the Gallimore Funeral Home. By mistake, Mrs. Hutslar left the car in automatic drive. As she walked in front of the machine her companion stepped on the foot ac-

Warren county's sixth traffic

Hutslar down and ran over her. In her excitement, Mrs. Haase put the car in reverse and the car passed over her again. Rushed to Mercy Hospital in Hamilton, she was reported dead on arrival.

The body was removed to Littleton Funeral Home in Springfield. Her nephew is Luther Doughman assistant police chief of that city.

Irwin, Ansel
9 Dec 1954

Services Today For Ansel Irwin

Ansel Irwin, aged 57, well known Mason resident, died Monday at his home following an illness of one year. He had been associated with Muennich Motors until recently.

Funeral services will be held at 2 p. m. Thursday at Gallimore Funeral Home with burial in Rose Hill Cemetery.

Surviving are his wife, Lucille, two daughters, Mrs. Eleanor Moler, Florida; Mrs. Pearl Bryan, near Mason; two sons, Glenn and Clifford Irwin of Mason and a brother, Will Irwin of Mason. Friends are asked to omit flowers and contribute to the Warren County Cancer Society.

Ivins, Mary Randall
26 Aug 1954

Mrs. Mary R. Ivins Services Monday

Mrs. Mary Randall Ivins, aged 82, died Friday night at Champion Rest Home here after an extended illness. She was a life-long resident of Lebanon and the widow of C. Wilbur Ivins, widely-known Lebanon druggist.

Funeral services were held Monday at the convenience of the family at Oswald Funeral Home with burial in Lebanon Cemetery.

Surviving ar a son, T. Randall Ivins and three grandsons.

Mabel Ann Ivins

19 AUG 1970

Mrs. Mabel Ann Ivins, 84, died Aug. 12 at her residence, Lebanon, Route one.

She is survived by six daughters, Mrs. Grace Bannest and Mrs. Dorothy Sieler, both of Lockland; Mrs. Catherine Jones, Mrs. Esther Carey and Mrs. Martha Jean Dunn, all three of Lebanon, and Mrs. Helen Hauck of Sardinia. Also surviving are four sons, Charles, Leroy and Clayton, all of Lebanon, and Edward of Lockland. Other survivors include 31 grandchildren and 22 great grandchildren.

Funeral services were held Saturday at the Oswald Funeral Home with burial in Bethany Cemetery.

9 DEC 1970

Rites Saturday For John James

John E. James, 70, of 445 South East Street, Lebanon, died early Wednesday at Middletown Hospital. He was a retired Lebanon meat market operator and for the past several years was custodian at the First Baptist Church.

Surviving are the wife Ruth; a son, Dick, and a brother, Herschel of Lebanon. Funeral services will be Saturday at 2 p.m. at Oswald Funeral Home. Friends may call from 6 to 9 p.m. Friday. Interment will in Lebanon Cemetery.

John E. James

16 DEC 1970

John E. James, 70, of 445 South East St., Lebanon, died last Wednesday in Middletown Hospital.

He is survived by his wife, Ruth; one son, Dick; and a brother, Herschel, all of Lebanon.

Funeral services were held at 2 p.m. Saturday at Oswald Funeral Home in Lebanon. Burial was at Lebanon Cemetery.

14 JAN 1970

Stella Jameson

Mrs. Stella Jameson, 93, of 111 Mound St., Lebanon, died on Jan. 12 at Miami Valley Hospital in Dayton. She was a lifetime member of the Lebanon Presbyterian Church.

Funeral services will be conducted at 2 p.m. today (Wednesday) at the Oswald Funeral Home with burial in the Lebanon Cemetery.

Mrs. Jameson is survived by two daughters, Mrs. Clark Weamer and Mrs. Cecil Griest both of Lebanon, one granddaughter, and three great-grandchildren.

Harold B. Janney

20 MAY 1970

Funeral services and burial for Harold Bradden Janney, former Warren County resident, were held May 7 at Dayton. Age 70, he died there May 5. Surviving are the wife, Mary Sophia Heaghman Janney; a daughter, Mrs. Barbara K. Sloan of Oakwood, and five grandchildren. A son James died two years ago.

Elsie Johnston

22 JULY 1970

Elsie K. Johnston, 74, of Route 1, Lebanon, passed away July 18 at the West View Nursing Home. She is survived by a daughter, Mrs. Marlin Watkins, of Lebanon; a son, Paul W., of Winchester; and four grandchildren.

Private burial services were held at 10:30 a.m. this morning (Wednesday) at Oswald Funeral Home, Lebanon, with interment at Spring Grove Cemetery, Cincinnati.

Henry Johnston

22 JULY 1970

Henry Johnston, 59, Route 1 Morrow, passed away at Epp Memorial Hospital July 21. Funeral services will be held Thursday at 2 p.m. at Vale Funeral Home, with interment at South Lebanon Cemetary. Visitation will be today (Wednesday) from 6-9 p.m.

He is survived by a son James, Route 1, Morrow; a daughter, Marlin Kersey, Dayton, two grandchildren; three brothers, Charles of Clarksville, Jess of Westboro, and Howard of Wilmington; One sister, Neva Runyan, of Oregonia.

Letcher Johnson

9 DEC 1970

Services for Letcher Johnson, 71, of 1262 Helaine Dr., Franklin, formerly of 8598 S. Union Road, Miamisburg, will be held at 2 p.m. today at the Ungleby Funeral Home, with the Rev. John Swint officiating. Burial will be in Miami Valley Memory Gardens.

Mr. Johnson died Saturday at Middletown Hospital.

Surviving are his widow, Rose; two sons, Forrest of Franklin, and Samuel of Redondo Beach, Calif.; a brother, Fred of Franklin; a sister, Mrs. Ollie Stamper of Morris Fork, Ky.; and four grandchildren.

Joe S. Johnston

Funeral services were held Monday for Joe S. Johnston, 78, of 13 Wilson Road, Fairborn.

Formerly of Waynesville, Johnston died Friday at his residence. 22 DEC 1970

He was a retired employe of National Cash Register and a member of the Krypton's Kentucky Lodge, number 905 F & AM.

He is survived by his wife, Ollie; two daughters, Mrs. Flossie Payne of Phillipsburg and Mrs. Amanda Carwile of Dayton; three sons, John W. of Springfield, Chester L. of Dayton and Denman G. of Texas; one bother, Wesley of Richmond, Ky; 12 grandchildren, seven great grandchildren and several nieces and nephews.

Funeral services were held at 2 p.m. at Seven Pines, Ky. Burial was at Seven Pines Cemetery. Stubbs-Conner Funeral Home was in charge of arrangements.

Paul Elton Johnston

18 FEB 1970

Paul Elton Johnston of 909 Snider Road, Mason, died February 12 at Christ Hospital in Cincinnati. He was 74 years old.

Mr. Johnston is survived by a sister, Anna McKibben of Dayton, and a brother, Homer Johnston of Snider Road.

Funeral services were held on Saturday at 1 p.m. at the Shorten Funeral Home in Mason. Burial was in the Monroe Presbyterian Cemetery at Nicholsville.

Lester Hulbert
5 JUNE 1985

Lester J. Hulbert, 88, of 4999 Waynesville Road, Oregonia, died Saturday, June 1, at Grandview Hospital, Dayton.

He was a retired carpenter and a member of the Carpenters' Local Union.

He was preceded in death by his wife, Abbie Alma.

He is survived by one daughter, Sue Kilburn of Miamisburg; one grandson; and one nephew.

Funeral service was held Tuesday, June 4, at the Stubbs-Conner Funeral Home in Waynesville. Burial was in Evergreen Cemetery, West Carrollton.

Leaola Hursh
17-APR. 1985

Leaola Hursh, 86, formerly of Middletown and Lebanon, died Friday, April 5, at Bethesda Hospital, Cincinnati.

She was preceded in death by her husband Lewis Hursh Sr. in 1952.

She is survived by two daughters, Elaine Jordan of Darien, Conn., and Mildred Crane of Lebanon; two sons, Robert Lee Sr. of Cincinnati with whom she made her home for the past 24 years, and Lewis Hursh Jr. of Springboro; 14 grandchildren; and 18 great grandchildren.

Funeral services were held Tuesday, April 9, at Vorhis Funeral Home, Middletown. Interment was in Woodside Cemetery, Middletown.

Randall Ivins
13-MAR. 1985

T. Randall Ivins, 80, formerly of Lebanon, died Wednesday, March 6, in Frederick, Md., where he lived.

Mr. Ivins operated Ivins Pharmacy in Lebanon from 1950 to 1971. After his retirement, he worked as a substitute teacher in the Lebanon schools. He was a member of the Lebanon Elks Lodge 422 and the Lebanon Masonic Lodge 26.

He is survived by three sons, Thomas of Middletown, Charles of California and Bruce of Frederick, Md.

Services were held Monday, March 11, at the Oswald Funeral Home, Lebanon. Rev. William Johnson officiated. Burial was in Lebanon Cemetery.

Memorial donations can be made to the American Heart Association.

Arnold Iames
16-OCT. 1985

Former Warren County resident Arnold W. Iames, 67, of Kettering, died Sunday, Oct. 13, at home.

Iames was a member of the Lebanon Eagles Lodge and the Lebanon VFW.

He also was a member of the South Park United Methodist Church, Dayton, and the John Durst Scottish Rite. He retired in 1984 from Alemite Co., where he was a district sales manager.

Survivors include his wife, Catherine; a daughter, Barbara Lincoln of Kettering; a brother, Earl Emerick of Hot Springs, Ark.; and two grandchildren.

Services will be at 10:30 a.m. Thursday, Oct. 17, at the Routsong Funeral Home, Oakwood and Irving avenues, Oakwood, with the Rev. Walter Custer officiating.

Calling hours will be from 5 to 9 p.m. today (Wednesday, Oct. 16) at the funeral home, with Masonic services at 7 p.m.

Burial will be in David's Cemetery, Kettering.

Lena Iorns
11 Sept. 1985

Lena M. Iorns, 92, East U.S. 22-3, Morrow, died Tuesday, Sept. 3 at Bethesda Care Warren County following a traffic accident.

Born in Lebanon, she was a member of the Lebanon Presbyterian Church and a charter member of the Warren County Historical Society.

Survivors include a son, Herschel of Williamsburg, Ky.; a sister, Lucy Settlemire of Xenia; and one granddaughter.

She was preceded in death by three sisters, Helen Chamberlain, Etta Nixon, and Grace Middleton, and a brother, Walter.

Services were held Friday, Sept. 6, at the Lebanon Presbyterian Church with the Rev. William Johnson officiating.

Contributions may be made to either the Warren County Historical Society or the Lebanon Presbyterian Church.

The Oswald Funeral Home, Lebanon, was in charge of arrangements.

Mollie Jackson
4 SEPT. 1985

Mollie Jackson, 97, died Sunday, Sept. 1, at the Quaker Heights Nursing Home, Waynesville.

Survivors include three daughters, Gladys Elkins, Frieda Petty and Fon Eva Brown; two sisters, Matilda Owens and Nettie Statton; and 20 grandchildren and 31 great-grandchildren.

Services were held Wednesday, Sept. 4, at the Breitenbach Funeral Home, Middletown. Burial was in Woodside Cemetery, Middletown.

Nora Jadwin
17 JULY 1985

Nora N. Jadwin, 94, of the Otterbein Home, near Lebanon, died there Wednesday, July 10.

A memorial service will be held at the convenience of the family.

Chauncey Jeffers
20-FEB. 1985

Rev. Chauncey E. Jeffers, 95, of the Otterbein Home, near Lebanon, died Wednesday, Feb. 13, at Middletown Regional Hospital.

Rev. Jeffers was a graduate of Taylor University, Wesley Theological Seminary. He was for 70 years a minister in the Methodist Protestant, the Methodist, and the United Methodist churches. For the past seven years, he was active in volunteer service at the Otterbein Home.

He is survived by his wife, Emma Jeffers; two sons, Glen of Fostoria and Harold of Chester Hill; two daughters, Mrs. Rosemary Osborn of Newark and Mrs. Miriam Wagner of Dayton; 16 grandchildren and 14 great-grandchildren.

Memorial services will be held Saturday, Feb. 23, at the Otterbein Home Chapel at 2 p.m. Rev. William McOmber will officiate. Burial will be in Otterbein Cemetery.

Thomas Hutchinson

FRANKLIN — Former Franklin resident Thomas Joseph Hutchinson, 69, of Fort Myers, Fla., died Friday evening at North Fort Myers Hospital.

Born in Middletown, he worked as executive secretary of the Building Trade Council of Dayton. In 1982, he retired and moved to Florida.

He is survived by his wife, Ida; his mother, Mrs. Margaret Hutchinson of Middletown; a son, Walter Thomas, of Franklin; one daughter, Mrs. Peggy York of Terre Haute, Ind.; one brother, William of Middeltown; two sisters, Sister Ann Hutchinson of Indianapolis, and Mrs. Mary Webb of Clearwater, Fla.; and five grandchildren.

Mass of Christian Burial will be Wednesday at 10 a.m. at the Holy Trinity Church in Middletown, the Rev. George Klein officiating. Burial will follow at Woodside Cemetery. Visitation will be Tuesday from 7 to 9 p.m. at the Wilson-Schramm Memorial Home in Middletown.

Memorial contributions may be sent to the American Cancer Society, asbestos research.

Milton Ingles

LEBANON — Milton Ingles, 76, of Celina, formerly of Lebanon, died at his home Thursday.

He was preceded in death by his wife, M. Jeanette, in 1983.

Survivors include a daughter, Mrs. Jo Anne Adkins of Celina, three grandchildren and a great-grandchild.

Funeral services will be at 11 a.m. Monday at the Oswald Funeral Home here.

Burial will follow at Miami Valley Memory Gardens.

Visitation will be Sunday from 2 to 5 and 7 to 9 p.m. at the funeral home.

Louis Jeffery

Louis (Bud) Jeffery, 66, of 44 Skokiaan Drive, Franklin, died Nov. 17 at Middletown Regional Hospital.

He retired from Stone Container, Franklin in 1975. He was a veteran of World War II.

Survivors include his wife, Phyllis; one son, Tracy, at home; daughters, Mrs. Diana Love of Webster, Fla. and Mrs. Debbie Moore of Franklin; five brothers, Charles of Harrison, James of Carlisle, Larry of Franklin, Robert and Merle, both of Palmetto, Fla.; three sisters, Mrs. Mildred Morton of Bradenton, Fla., Mrs. Margaret Williams of Madison, Fla. and Mrs. Clara Price of Glendale, Calif.; two granddaughters.

Services will be held Thursday, Nov. 21 at 2 p.m. at the Unglesby-Anderson Funeral Home, Franklin, with the Rev. James Maggard officiating. Burial will be in Springboro Cemetery. Visitation will be Wednesday, Nov. 20 from 4 to 9 p.m. at the funeral home.

T. Randall Ivins

LEBANON — T. Randall Ivins, 80, of Frederick, Md., a former longtime Lebanon resident, died Wednesday at Homewood Retirement Center in Frederick where he had resided the past year.

He operated the Ivins Pharmacy here from 1950 to 1971 and was a substitute teacher in Lebanon schools after retirement. He was a member of the Lebanon Elks Lodge 422 and Lebanon Masonic Lodge 26.

He is survived by three sons, Thomas of Middletown, Charles W. of California and Bruce of Frederick, and three grandchildren.

Services will be at 11 a.m. Monday at the Oswald Funeral Home. Burial will be in Lebanon Cemetery. Visitation will be Monday one hour before services at the funeral home.

Gary A. Jones

FRANKLIN — Gary A. Jones, 24, of Franklin, died Wednesday at the University of Cincinnati Hospital of a brain tumor. The funeral home would not disclose his address.

Mr. Jones was a test technician for Reynolds and Reynolds Computer Co.

He is survived by his wife, Sandra; son, Troy; father, James F. Jones of Cincinnati; mother, Mrs. Rita A. Stewart of Cincinnati; grandparents, Mr. and Mrs. A.T. Jones; and great-grandfather, Roy.

Funeral services will be at 11 a.m. Saturday at the Forest Dale Church of Christ, 604 W. Kemper Road, Springdale, the Rev. Jerry Mueller officiating. Burial will be in Rest Haven Cemetery. Visitation will be at the church one hour before services.

Arrangements are by the Vorhis Funeral Home in Springdale.

Contributions may be made to the Hospice of Middletown.

Lewis Johnson

Lewis Johnson, 63, 2720 Factory Road, Springboro, died March 8, at Kettering Medical Center.

He is a native of Kentucky and was retired from Dayton Walther Corp.

Survivors include his wife, Gloria; two daughters, Mrs. Gloria Littleton of Taylor, S.C. and Mrs. Gail O'Neil of Billings, Mont.; one son, Todd of Springboro; seven sisters and one brother and four grandchildren.

Funeral services were held Monday, March 11 at Sanner Funeral Home, West Carrollton, with the Rev. Delbert Dawes officiating. Burial was in Springboro Cemetery.

John Paul Jones

John Paul Jones, 76 of 1030 Dale Ave., Franklin, died Jan. 21 at Grandview Hospital, Dayton.

He is survived by his wife, Betty; son John Jones of Dayton; three daughters, Mrs. Linda Dalton of Carlisle, Mrs. Susan Craycraft of Franklin and Mrs. Louise Hall of Michigan; two brothers, Arthur of Missouri and Earl of Zanesville; two sisters, Mrs. Rose Burden of McComb and Mrs. Lucille Atrash of Findlay; 18 grandchildren and three great-grandchildren.

Funeral services will be held Thursday, Jan. 24 at 1 p.m. at the Unglesby Funeral Home, Franklin. Visitation will be Wednesday, Jan. 23 from 4 to 9 p.m. at the funeral home. Burial will be at Woodhill Cemetery, Franklin.

Neal Johnson

Mr. Neal Johnson, 59, 230 Walnut Ave., died Monday, Nov. 18, at Kettering Medical Center where he had been a patient for two weeks. He had been ill for a long period of time.

Mr. Johnson was born in Madison County, Ky., and had lived in the Carlisle area for most of his life. He had worked as a foreman at the Valley Sheet Metal Co. in Middletown. He held the rank of sergeant in the U.S. Army during World War II and was a member of Bethany Baptist Church.

His survivors include his wife, Thelma; five sons, Fred and Terry of Carlisle and Kenny, Ted and Everett of Middletown; his mother, Mrs. Randa Johnson of Waco, Ky.; four brothers, Willard of Waco, Lawrence of Glendale, Oh., Russell of Clearwater, Fl., and Harold of Middletown; and two sisters, Mrs. Bonnie Morton of Nicholsville, Ky., and Mrs. Doris Turpin of Richmond, Ky.; and five grandchildren.

Services were held Friday at the Unglesby-Anderson Funeral Home with the Rev. Loma Gwinn officiating. Burial will be in Springboro Cemetery.

Elvis W. Igo 6 MAY 1996

Elvis W. Igo, 73, of 6965 Franklin-Madison Road, Middletown, died at 3 p.m. Sunday, May 5, 1996, at his residence.

Mr. Igo

Born in Morehead, Ky., on April 7, 1923, he lived here most of his life. Mr. Igo was employed in the factory at Inland Container Corp. for 32 years. He retired in 1984. He was a member of the Full Gospel Outreach Pentecostal Church of God.

Surviving are his wife of 53 years, Carrie J.; three sons, Eddie LeRoy Igo of Franklin and Richard and Jeffrey Igo, both of Middletown; a daughter, Linda S. Nolen of Franklin; eight grandchildren; two great-grandchildren; a brother, Harry of Toledo; and a sister, Betty Jane Cecil of Morehead, Ky.

He was preceded in death by three brothers, Leonard, Buck and Roy Igo; and a sister, Myrtle Carroll.

▼ **ARRANGEMENTS:** Funeral services are set for 2 p.m. Wednesday at the Joseph R. Baker Funeral Home, with the Rev. James Maggard officiating. Burial will be at Woodside Cemetery.

Gladys Ivins 24 JAN 1996

Gladys Hazel (Gum) Ivins, 89, of Lebanon for most of her life, died Monday, Jan. 22.

She was born in Marlinton, W.Va. on April 7, 1906 to Harry D. and Woodsie Frances (Ratliff) Gum. She retired in 1973 after 29 years as a secretary for the Warren County Board of Education and was a member of the Lebanon United Methodist Church and the Lebanon Community Club.

She was preceded in death by her parents; and husband Charles on Oct. 26, 1994.

Visitation will be Thursday, Jan. 25 from 5-7 p.m. with funeral service Friday, Jan. 26 at 10 a.m. all at Oswald-Hoskins Funeral Home, Lebanon with Rev. James Ludwick officiating. Burial will follow in Lebanon Cemetery.

Freda B. Inloes 9 FEB - 1996

CHAUTAUQUA — Mrs. Freda Blanche Inloes, 80, of 10041 Elanja Drive, Miamisburg (Chautauqua), died at 7:10 a.m. Thursday at the Barbara Parke Care Center in Middletown.

A homemaker, she was born on March 1, 1915, in College Corner, Ohio, and formerly resided in Oxford.

Mrs. Inloes is survived by her husband of 54 years, Louis H.; three sons, Bob of Fairfield, Ed of Carlisle and Gerald of San Antonio, Texas; a daughter, Martha L. Reese of Miamisburg; two sisters, Hilda Agnew of Eaton and Patty Master of Oxford; seven grandchildren, Juli Reese, Diana Reese Clary and David, Nichole, Jerry, Stephen and Christopher Inloes; and two great-grandchildren.

She was preceded in death by two grandchildren, Susan Reese in 1992 and Brett Inloes in 1977.

Funeral services will be at 2 p.m. Saturday at the Unglesby-Anderson Funeral, 1357 E. Second St., Franklin, with the Rev. Ted Holstein officiating. Burial will be in Butler County Memorial Park.

Charles Innis 21 FEB - 1996

Charles Franklin Innis, 80, of Kings Mills, died Monday, Feb. 19 at Bethesda North Hospital.

He was born in 1915 in Maineville and had been employed in machine repair with Ford Motor Co. He was an Army veteran.

He was preceded in death by his parents Lulu Mae (Kendle) and Lucian Innis.

He is survived by wife Miriam (Boger) Innis; sisters Mildred Holden of Urbana and Janet McDaniel of Lebanon; brothers John of Dayton, Robert of Blue Ash and Lester of Montgomery; and many nieces and nephews.

Funeral service was held Wednesday, Feb. 21 at 1:30 p.m. at Tufts Schildmeyer Family Funeral Chapel in Loveland with burial at Hopkinsville Cemetery.

James Innis 7 JAN 1998

James Robert "Bob" Innis, 84, a native of Maineville, died Friday, Jan. 2 at Blue Ash Nursing Home.

He was a graduate engineer of the University of Cincinnati and was retired from Coulter Electronics Inc. of Hialeah, Fla.

He was Past Patron of Fairmount Chapter 352 OES, Past Master of Excelsior Lodge 369 F&AM, member of the Price Hill Chapter 164 RAM, McMillan Lodge 141 F&AM, Valley of Cincinnati Ancient Accepted Scottish Rite, and was a member of the Crystal River, Fla. United Methodist Church.

He was preceded in death by his wife of 53 years, Jean Blum Innis; parents Lucian and Lulu Mae Innis; brothers Howard L. and Charles F. "Tad" Innis; sisters-in-law Bette B. Innis and Miriam B. Innis; and brother-in-law Robert McDaniel.

He is survived by son James R. Jr. and daughter-in-law Carolyn C. of Harrison; grandchildren Julia G. of Baltimore, Md. and Robert P. of San Francisco; sisters Mildred Holden and husband Travis of Urbana and Janet McDaniel of Lebanon; brothers Lester and wife Ruth B. of Montgomery and John William "Joe" of Dayton; brother-in-law Walter Blum Jr. and wife Virginia S. of Maineville; and 18 nieces and nephews.

Funeral service was held Tuesday, Jan. 6 at Paul R. Young Funeral Home, Mt. Healthy with visitation Monday, Jan. 5.

Burial was at Baltimore Pike Cemetery, Cincinnati. Fairmount 352 OES services were held Monday.

b. 18 JUL 1913

Dick Irelan 1998

Dick A. Irelan, 86, of Waynesville died Saturday, Dec. 19 at Otterbein-Lebanon.

He graduated from Steele High School in Dayton and Ohio University in Athens, where he was a Beta Theta Pi.

He was a member of the First Church of Christ Scientist, Centerville, Sons of the American Revolution Montgomery Chapter, Dayton, Dayton Masonic Lodge 147 F& AM, Scottish Rite, York Rite and Antioch temple all in Dayton, OES Chapter107 in Waynesville, the Warren County Shrine Club, the Little Miami River Association (charter member) and past president and grand marshal of the Warren County Fair Board.

He was preceded in death by his sister Wilma Knowlton.

He is survived by his wife Lutie M.; four daughters and sons-in-law Patricia A. and Mark Mason of Waynesville, Lynne M. and Kent Lindsey of Chicago, Deborah J. and Gary Harlow of Beavercreek and Mary Beth Irelan of Georgetown, Ky.; grandchildren Victoria Williams, Jennifer Miroballi, Vance Vair, Gregg Harlow, Jeff Lindsey, Grant Harlow, Tiffany Wilson-Mobley, Vanessa Woods, Hailey Beth Wilson and Layne Peden; nine great-grandchildren; and brothers Frank of Texas and Patrick of Waynesville.

Funeral service was Tuesday, Dec. 22 at Stubbs-Conner Funeral Home, Waynesville.

If desired, contributions may be made to Shriners Hospitals or the First Church of Christ Scientist, Centerville.

Clayton Ivins 1998

8

Clayton Blake Ivins, 85, of Lebanon died Saturday, Dec. 5.

He was born Nov. 1, 1913 in Tulahoma, Tenn. to Harry L. and Mabel (Blake) Ivins. He was a World War II U.S. Army veteran. He worked for both Warren County and Turtlecreek Township as a mechanic before retiring from Doebler Brothers Inc. in 1978. He was a member of the Bethany United Church of Christ.

He was preceded in death by his parents; brothers Charles, Leroy, Edward and Robert; and sisters Dorothy, Grace and June.

Survivors include his wife of 52 years, Shirley (St. John) Ivins of Lebanon; sons Richard of Cincinnati and Randy and his wife Cyndi of Evendale; daughter Gail and her husband Jeff Clark of Cincinnati; sisters Jean Dunn and Catherine Jones both of Lebanon, Esther Carey of Kissimmee, Fla. and Helen Hauck of Hamden, Ohio; and grandchildren Rachael and Nicholas Ivins.

Visitation was Tuesday, Dec. 8 with funeral service Wednesday, Dec. 9 all at Oswald-Hoskins Funeral Home, Lebanon with Pastor Allen Wentworth officiating. Interment was at Bethany Cemetery.

Memorials are requested to the Alzheimer's Association, 644 Linn St., Suite 1026, Cincinnati, Ohio 45203.

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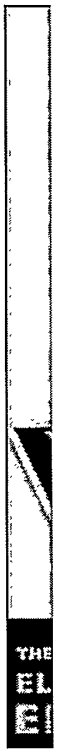

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The following investigation was conducted by Special Agent [redacted] on 06/28/2005:

Writer accessed www.uspto.gov, operated by the United States Department of Commerce, Patent and Trademark Office. Using the search engine at www.uspto.gov, a search was conducted for all granted patents and applications that listed "Ivins" as an inventor. The database searched included all patents since 1976. The search engine revealed two patents listing Ivins, Bruce on the inventor line. These patents are as follows:

1) "Asporogenic B anthracis expression system," invented by [redacted] Ivins, Bruce (Frederick, MD). The patent was originally filed on November 23, 1994 and was granted on November 13, 2001 under the patent number 6,316,006.

2) "Method of making a vaccine for anthrax," invented by Ivins; Bruce (Frederick, MD); [redacted] The patent was originally filed on March 7, 2000 and was granted on May 14, 2002 under the patent number 6,387,665.

A search was also conducted for [redacted] in the above mentioned website. The only result returned from this search was the patent labeled [redacted] mentioned above. Based on this search, it appears as if [redacted] only has one patent under his name.

Printouts from the abovementioned searches for each of the patents are attached to and made part of this document.

Administrative: The abovementioned search was performed in response to emails found during a review of electronic evidence gathered during the investigation into Major Case 184. The emails in question were authored by BRUCE IVINS on April 15 and 16, 1999. The emails discussed the process involved with filing the patent. Copies of these emails are attached to and made part of this document.

[redacted]

[redacted]

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United States Patent **6,387,665**
Ivins, et al. **May 14, 2002**

Method of making a vaccine for anthrax

Abstract

A method of making a vaccine for anthracis that inolves a bacterial expression system and production and use of protective antigen (PA) against Bacillus anthracis. The PA immunogen is useful in a vaccine against human anthrax. The PA can be produced by an asporogenic organism which produces the desired antigen, which is then harvested from the supernatant.

Inventors: **Ivins; Bruce** (Frederick, MD); **Worsham; Patricia** (Jefferson, MD); **Friedlander; Arthur M.** (Gaithersburg, MD); **Farchaus; Joseph W.** (Frederick, MD); **Welkos; Susan L.** (Frederick, MD)

Assignee: **The United States of America as represented by the Secretary of the Army** (Washington, DC)

Appl. No.: **520215**

Filed: **March 7, 2000**

Current U.S. Class: **435/71.1**; 424/184.1; 424/234.1; 424/246.1; 435/69.1; 435/69.4; 435/252.3; 435/252.31; 435/320.1; 435/485; 530/350

Intern'l Class: C12P 021/04

Field of Search: 424/184.1,234.1,246.1 530/350 435/69.1,69,71.1,320.1,172.1,172.3,252.3,252.31,200.1

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Primary Examiner: Graser; Jennifer E.
Attorney, Agent or Firm: Arwine; Elizabeth, Moran; John Francis, Harris; Charles H.

Parent Case Text

This application is a divisional application of Ser. No. 08/346,238 filed Nov. 23, 1994, said application allowed.

Claims

What is claimed is:

1. A method of making a vaccine comprising: incorporating a protective antigen produced by recombinant asporogenic B. anthracis with a pharmaceutically acceptable carrier, wherein said recombinant asporogenic B. anthracis was isolated from a .DELTA.Sterne-1(pPA102) strain of bacteria and said recombinant asporogenic B. anthracis does not have the ability to bind a dye when grown on Congo Red Agar.

2. The method of claim 1, wherein the recombinant asporogenic B. anthracis is B. Anthracis .DELTA.Sterne-1(pPA 102)CR4.

3. The method of claim 1, wherein the vaccine is in the form of a suspension.

4. The method of claim 1 wherein the vaccine is in the form of buffered suspension.

5. The method of claim 1 wherein said carrier is an adjuvant.

Description

FIELD OF THE INVENTION

This invention relates to the bacterial expression system, production and use of protective antigen (PA) against Bacillus anthracis. The PA immunogen is useful in vaccine against human anthrax. The PA can be produced by an asporogenic organism which overproduces the desired antigen, which is then harvested from the supernatant.

BACKGROUND OF THE INVENTION

Bacillus anthracis is the etiologic agent responsible for anthrax, a disease often found in persons exposed to infected animals or their products. Persons particularly exposed to animals include veterinarians, laboratory technicians, ranchers and employees working with skin or hair of animals. The mode of entry into the body may be the skin or, when contaminated meat is eaten, the gastrointestinal tract. Inhaling of spores can cause inhalation anthrax, a disease that can be fatal. Vaccines against Bacillus anthracis have been available. Virulent strains of the organism produce two toxins and a poly-D-glutamic acid capsule which are coded for on two endogenous plasmids, pX01 and pX02, respectively. Loss of either of the plasmids results in an attenuated strain of reduced virulence, while loss of both results in an avirulent organism. The history of the USAMRIID Sterne strain of B. anthracis prior to 1981 is uncertain, though it is believed to be derived from the Sterne strain isolated at the Onderstpoort Research Laboratory in Pretoria, South Africa.

In 1985 the Bacillus anthracis protective antigen (PA) gene was cloned into a plasmid (pUB110) resulting in the formation of a recombinant plasmid identified as pPA102, which was reported in the literature (Ivins and Welkos, Infection and Immunity, 54:537-542 (1986)). The production of vaccines lacking lethal factor was possible thereby. However, a primary problem remained, since the Bacillus anthracis formed spores. Once spores have formed, they persist in the environment for months and years. Once the laboratory environment contains such spores, it is very difficult to free the environment of the spores.

It was also previously reported that protective antigen (PA) could be produced in baculovirus. [Iacono-Connors, et al., Infection and Immunity, 58:366-372 (1990); Iacono-Connors, et al., Infection and Immunity, 59:1961-1965 (1991)] A major problem in production of the PA in the baculovirus disclosed therein is that the desired antigen requires a complex purification process. Even after purification by immuno-affinity chromatography, undesired cellular material continues to contaminate the desired product.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention provides organisms which produce protective antigen (PA) lacking lethal factor and edema factor proteins which, when present as contaminants in vaccine, can cause serious side

effects. The producing organisms of the invention are also, surprisingly, non-sporulating. Furthermore, the desired antigen is expressed into the supernatant. Hence, the protective antigen produced is easily purified and, though protective, does not cause many of the troublesome side effects of prior art vaccines. The organisms of the invention lacking spore-forming function may be killed by heat shock at temperatures as low as 60.degree. C. for 60 minutes. Hence, contamination of the environment with viable spore-forming organisms is easily avoided and decontamination is easily accomplished.

Genesis of .DELTA.Sterne-1(pPA102)CR4:

A 6 kb Bam HI fragment harboring the PA structural gene isolated from the endogenous Sterne plasmid pXO1 was ligated into plasmid pBR322 and cloned into Escherichia coli bacteria (Vodkin and Leppla, 1983). From the resultant recombinant plasmid pSE36, the 6 kb fragment was then subcloned into the gram-positive vector pUB110 using the Bam HI restriction site. The resulting plasmid was transformed into B. subtilis IS53 and two stable PA producing, kanamycin resistant isolates were found (pPA101 and pPA102) (Ivins and Welkos, 1986). Subsequent analysis of the plasmids revealed that both had suffered spontaneous deletions. The pPA102 was found to have lost 4.2 kb of DNA from 363 bp 3' of the kanamycin resistance gene to approximately 164 bp 5' of the start of the PA structural gene, a result consistent with the observed inactivation of the phleomycin resistance gene of pUB110. The plasmid was then electrotransformed into .DELTA.Sterne-1, a plasmid-free strain of B. anthracis (Infection and Immunity, 52:454-458 (1986) and transformants were selected for kanamycin resistance. Transformants displaying a stable PA+, kanamycin resistant, (LF-, EF-, capsule-) phenotype were selected. This strain, .DELTA.Sterne-1(pPA102), was then subjected to Congo Red agar selection for mutants displaying an inability to bind the dye, a characteristic known to correlate with an asporogenic phenotype (Worsham, submitted). The selected isolate, now designated .DELTA.Sterne-1(pPA102)CR4 was further subcultured three times to insure that a single clone was isolated. This clone has served as the seed stock for all research and development of fermentation conditions, and purification of PA.

Materials and Methods:

Fermentation Conditions

Media: FA medium was used for all plates and liquid cultures described here unless otherwise specified. FA medium consisted of 33 g/l tryptone (Difco), 20 g/l yeast extract (Difco), 2 g/l L-histidine, 8 g/l Na2HPO4, 7.4 g/l NaCl, 4 g/l KH2PO4 adjusted to pH 7.4 with NaOH.

Precultures: A working stock of .DELTA.Sterne-1(pPA102)CR4 was prepared from the seed culture by streaking cells on an FA medium plate containing 40 .mu.g/ml of kanamycin. A sweep from the confluent growth zone on plate was cultured one time in liquid FA medium supplemented with kanamycin 40 .mu.g/ml to a final O.D..sub.600nm of 4.0. This culture was checked for purity by streaking on SBA plates, and diluted into multiple vials containing sterile 100% glycerol to a final glycerol concentration of 50% (v/v). These stocks were stored at -70.degree. C. A single vial was removed at the start of each fermentation cycle and discarded after use. The defrosted cells were streaked onto FA plates containing 40 .mu.g/ml kanamycin and incubated at least 16 hrs at 37.degree. C. After 16 hrs the plated cells were used to inoculate 50 mls of FA medium supplemented with 40 .mu.g/ml kanamycin in a 250 ml baffled-Erlenmeyer flask (Bellco Laboratories). The culture was incubated at 37.degree. C. at 200 rpm for 6 hrs or until an O.D..sub.600nm of 4-6 was obtained. The cells were then subcultured into 50 mls of FA medium in an identical flask under identical conditions. After 6 hrs, or a culture O.D..sub.600nm of 6.2-6.5, a 1.6% (v/v) inoculum was transferred to 300 mls of PA medium supplemented with 40 .mu.g/ml kanamycin in a 2 liter baffled Erlenmeyer and incubated at 37.degree. C. at 200 rpm for 7 hrs, or until a final O.D..sub.600nm of 3.5-3.7 was achieved.

Fermentation conditions: The fermentations described here were carried out using a New Brunswick Bio-Flo 3000 equipped with a 5.0 liter working volume glass vessel and stainless steel headplate and hemispherical bottom cooling dish. Four liters of FA medium were added to the vessel, which had been previously completely disassembled, scrubbed in a dilute Envirochem solution and autoclaved for 15 min after the addition of 4 liters of H2O. The polarographic DO.sub.2 probe (Ingold) and pH probes (either liquid or gel filled, Ingold) were also inserted and all addition and sampling ports were sealed or clamped and wrapped in aluminum foil. Addition lines consisted of surgical grade autoclavable Tygon tubing (Thomas Scientific) and all lines were sealed with the exception of the condenser, which was left open to permit pressure release, but covered with aluminum foil. The vessel was autoclaved using a 10 min exposure time at 121.degree. C. and removed from the autoclave as soon as sufficient cooling had occurred to allow opening of the autoclave. The vessel was then immediately connected to the fermentor unit and the condenser line was connected to a sterile liquid trap and 0.2.mu. capsule filter to avoid the introduction of contaminants during the cooling process. The vessel was then cooled to 37.degree. C. using the fermentor driven temperature control and positive pressure was provided using compressed sterile filtered air. Once the vessel had cooled to 37.degree. C. sterile filtered kanamycin was added to a final concentration of 40 .mu.g/ml. The agitation was activated at 150 rpm and aeration was adjusted to 1-1.2 volume/volume/min (vvm) and antifoam C (DOW), that had been diluted 10-fold into H.sub.2 O and autoclaved, was added to a final concentration of 200 ppm.

A preinoculation sterility check was conducted for a minimum of 16 hrs during which time pH, agitation and temperature were continually monitored. After the 16 hrs required for DO.sub.2 probe polarization, the DO.sub.2 was also monitored along with turbidity. The D.sub.2 probe was calibrated using an INGOLD calibration device which sets the zero value to 4 mA and 100% to the oxygen tension determined by the solubility of oxygen in the medium after aeration and agitation at 37.degree. C. The calibration and response of the electrode was then checked by sparging with pure N.sub.2. The vessel was judged to be sterile if the pH and DO.sub.2 remained constant and no increase in turbidity was observed. It should be emphasized that the short autoclave cycle for vessel sterilization was required to minimize caramelization, Millard and other chemical degradation reactions which are problematic due to the high concentrations of yeast extract and tryptone in FA medium. As an additional confirmation of sterility, 50 mls was aseptically removed from the fermentor to a 250 mls Erlenmeyer and incubated at 37.degree. C. at 200 rpm for 48 hrs with no sign of growth. Under the conditions outlined here contamination has not been observed in more than 10 fermentation cycles.

Once the sterility of the vessel had been verified, the 300 ml inoculum described above was added to the vessel through the addition port of the headplate and the initial O.D..sub.600nm was recorded. A sample of the inoculum was also streaked on SBA plates and incubated for 48 hrs at 37.degree. C. to verify inoculum purity. Using the Bio-Flo 3000, aeration was maintained at 75% of saturation by increasing agitation from the initial 150 rpm to a maximum of 400 rpm and ultimately by supplementing the 1 vvm aeration rate with pure oxygen. The mixture rate and percentages of air and oxygen were controlled by a solenoid and algorithm developed by Nev Brunswick Scientific. Both gases had a working pressure of approximately 10 psi.

The O.D..sub.600nm dry cell weight (DCW), production of PA, DO.sub.2, pH, agitation and temperature were monitored throughout each fermentation cycle. The O.D..sub.600nm DCW and PA production analysis were carried out by manually sampling the fermentation liquor at hourly intervals using a sterile sampling port. O.D..sub.600nm was measured after dilution of the culture using sterile medium prepared for that fermentation. For each O.D.600 determination, two appropriate dilutions were made and results were considered acceptable only when both dilutions yielded a linear response. DCWs were determined starting with a 2 hr point by centrifuging 10 mls of fermentation liquor at 11,953.times.g for 10 min, resuspending the cell pellet in 10 mls of sterile PBS and pelleting the cells again under the same conditions. The cell pellet was resuspended in a minimal volume of PBS and

transferred quantitatively to a preweighted Eppendorf centrifuge tube and centrifuged at 14,000 rpm for 5 min. Excess PBS was removed and the cell pellet was dried in a speed-vac for 72 hrs under vacuum and a medium heat setting. A final analysis of the dry weight versus O.D..sub.600nm revealed that the relationship between the two parameters was adequately fit with a linear function.

Fermentation Reproducibility: The reproducibility of the cell growth parameters, biomass and PA production in fermentations carried out with the Bio-Flo 3000 under the conditions described above have been summarized in Table I below. Two fermentations were carried out at 75% of the maximum dissolved oxygen concentration in a strict batch mode with no pH control or additions other than antifoam C. The variation in the agitation rate during the first 100 min of the fermentation was the result of the AGDO.sub.2 (agitation DO.sub.2) control mode chosen to maintain the dissolved oxygen tension at 75% of the maximum. Briefly, this algorithm attempts to control the oxygen tension by first altering the agitation rate until this proves insufficient, at which point the process air is supplemented with pure oxygen as needed to maintain the desired DO.sub.2. The temperature was held constant at 37.degree.+/-0.1.degree. C. The pH was monitored, but not regulated as an internal check on the aeration of the vessel during the course of the fermentation. The fact that the pH revealed a decrease on only 0.2 pH units in the first 150 min was consistent with an aerobic culture metabolizing the limited carbohydrate supplied with the yeast extract to CO.sub.2 and organic acids. Once the carbohydrate was exhausted after ca. 150 min, the bacillus switched to the utilization of amino acids and peptides for a carbon source, which under aerobic conditions resulted in the release of NH.sub.4 OH and the observed increased culture pH.

These fermentations were sampled on an hourly basis and allowed to proceed until no further increase in O.D..sub.600nm was observed over two time points. O.D..sub.600nm, DCW analysis and product measurements were carried out for each sample as described above. Samples for PA production were sterile filtered followed by the addition of HEPES and the complete protease cocktail as described under PA quantitation. The samples were concentrated, desalted and ultimately concentrated 80-fold prior to being analyzed using SDS-PAGE. The major band of the gel corresponded to the 83 kDa PA product. An increasing in the intensity of the protein band was seen with increasing fermentation time. Study of a Western blot of another time course of a batch fermentation was developed with polyclonal rabbit anti-PA83. Comparison revealed that along with increasing PA 83 kDa there was also a pronounced increase in the abundance and form of proteolytic degradation products of PA.

TABLE 1

Summary of Aerobic .DELTA.Sterne-1(pPA102)CR4 Fermentations

Fermentation Conditions	Final Conc. (.mu.g PA83/ ml	Final Yield (mg PA83)	Final Yield (mg PA83/g DCW)	Specific Growth Rate	Doubling Time T.sub.D (min)
Aerobic, Batch	51	235	8.10	0.0132 min.sup.-1	53
Aerobic, Batch	64	301	10.7	0.0136 min.sup.-1	51
Aerobic, Batch pH constant	45	225	7.40	0.0136 min.sup.-1	51
Aerobic, Fed-Batch (non-continuous)	68	360	ND	0.0116 min.sup.-1	60

DCW = dry cell weight

The data presented in Table 1 demonstrated that the PA yield on a unit volume and biomass basis, as well as the cell growth parameters, were reproducible for the batch fermentations conducted without pH

control. The final fermentation pH values of 8.57 and 8.67 after an elapsed fermentation time of ca. 8 hrs were also comparable. The effect of prolonged exposure to these mildly alkaline conditions on cell growth, PA production and subsequent degradation was investigated by repeating the fermentation at a constant pH of 7.50+/-0.05 pH units. This was accomplished using the immersed vessel pH probe and automated additions of 2 N HCl or 1 N NaOH. The results shown in Table 1 demonstrate that there was no clear effect of constant pH on any of the parameters evaluated. SDS-PAGE analysis of the fermentation time points sampled for PA production also revealed no significant differences.

The final fermentation presented in Table 1 was a noncontinuous fed-batch trial during which 1/10 volume of a 10-fold concentrate of sterile-filtered tryptone was added after 5 hrs or an O.D..sub.600nm of 7.5. The result suggested that such fed-batch fermentations provide possible protocols for improvement to increase yield and decrease proteolysis.

Harvest conditions: Fermentations were allowed to proceed until no further increase in O.D..sub.600nm was observed. At this point, the fermentor was cooled to 10.degree. C. and the protease inhibitors phenylmethylsulfonyl fluoride (PMSF), 1,10-phenanthroline (OP) and ethylenediamine tetraacetate (EDTA) were added to final concentrations of 0.1, 0.05 and 2 mM, respectively. The cells were then pumped from the fermentor vessel at room temperature using an Amicon DC10L concentrator equipped with a 10-ft.sup.2 0.1 .mu.polysulfone hollow-fiber cartridge. The fermentor liquor was diluted 1:1 with 25 mM diethanolamine (DEA), 50 mM NaCl, 2 mM EDTA, 0.1 mM PMSF adjusted to pH 8.9 with HCl. The filtrate was collected at an operating pressure of less than 20 psi and transferred directly to a second Amicon DC10L equipped with two 30 kDa cutoff 10-ft.sup.2 wound spiral cellulosic cartridges. The filtrate was concentrated approximately 10-fold before being subjected to diafiltration at an operating pressure of less than 30 psi against the same buffer. The conductivity of the retentate was monitored with an Amber Sciences conductivity meter and platinum immersion pencil-type electrode. The diafiltration step generally required 20 liters of buffer, but was considered complete only after the conductivity of the concentrated retentate was equivalent to that of the starting buffer.

Quantitation of 83 kDa PA in crude fermentation liquor: The fermentation liquor was sampled using a sterile port at regular intervals throughout the fermentation process. The samples for PA determination were filtered through syringe type 0.2.mu. cellulose acetate filters, 0.1 mM PMSF, 2 mM EDTA, 50 .mu.M OP and 20 mM HEPES pH7.3 were added and the samples were frozen at -70.degree. C. The samples were defrosted on ice and concentrated using Amicon Centricon 30 concentrators at 4500.times.g. The samples were concentrated approximately 10-fold, diluted to the original volume with 10 mM TRIS pH8.0, 0.1 mM PMSF, 2 mM EDTA, 0.05 pM OP and concentrated again. The concentrated sample was desalted again using the same buffer, frozen and finally lyophilized using a Speed-Vac. The dried samples were dissolved in 25 .mu.l of the TRIS buffer described above and diluted 1:1 with a 2.times.SDS solubilization buffer consisting of 50 mM Na.sub.2 CO.sub.3, 4% (w/v) SDS, 12% (v/v) glycerol, 2% (v/v) 2-mercaptoethanol and 0.01% (w/v) Bromphenol Blue prior to heating at 95.degree. C. for 5 min. The fermentation samples containing varying amounts of PA 83 kDa were solubilized as described above and run on a Daiichi 4-20% gradient TRIS/TRICINE gel to approximate total yield of PA. Two hundred to 2000 ng samples of purified PA were solubilized in the same buffer and loaded onto the gel in constant total volume of 3 .mu.l. Three or four appropriate dilutions of the fermentation samples determined from the first gel were loaded onto the gel with the standards and electrophoresed at 100 V initially and 140 V once the samples entered the separating gel and until the Bromphenol Blue dye reached the bottom edge of the separating gel. The gel was then fixed in 10% (v/v) acetic acid 20% (v/v) MeOH for 10 min, rinsed with MQ H.sub.2 O and stained with Coomassie Brilliant Blue 0.05% (w/v) in 10% (v/v) acetic acid for a minimum of 16 hrs to allow complete and uniform staining. The stained gel was then destained in 10% (v/v) acetic acid until the background contained no visible residual dye. The gel was then scanned on a laser densitometer (LKB, Ultrascan XL Laser Densitometer). Representative portions of the gel without protein were randomly

chosen and scanned to determine background absorption for an accurate baseline. The region to be scanned for each lane containing PA was then visually aligned to insure that the entire protein peak and adequate baseline were included in each scan. The scans were completed and the integration values were determined using the LKB preprogrammed Gaussian algorithm and later were confirmed by cutting out individual peaks and manually integrating based on peak weight. The resulting integration values were plotted using Sigmaplot (Jandel). Linear regression of the results revealed typical r values of 0.992-0.996. The linear standard curve was then used to quantitate the amount of 83 kDa PA in the various fermentation samples based on the same integration methods.

Purification: The exact volume and conductivity of the PA in DEA buffer was determined and solid KCl was added to the solution to a final concentration of 30 mM and conductivity of 10-11 mmhos/cm. The PA was pumped with a peristaltic pump through a monoQ column prepared by collecting 100 mls of hydrated Bio-Rad Macro Prep 50Q on a sintered glass filter and washing sequentially with 1 liter of 25 mM DEA, 50 mM NaCl, 1 mM EDTA, 50 .mu.M OP and 0.1 mM PMSF pH8.9 and 1 liter of the same buffer with 30 mM KCl added. The conductivity (10-11 mmhos/cm) and pH of 8.9 of the eluate from the Macro Prep 50Q after the second wash were comparable to that of the PA solution after addition of KCl. The Macro Prep 50Q resin was then degassed and slurry packed into a Pharmacia K column with a Rainin Rabbit-Plus peristaltic pump at 48 rpm and a flow rate of 15 mls/min. The final column volume was (5.times.5 cm) 98 mls. The PA solution was pumped through the Macro Prep 50Q column at a rate of 10 mls/min and the eluate was collected until all of the PA sample volume was loaded and the column washed with an additional 100 mls of DEA/KCl buffer. The eluate containing unbound PA was concentrated and diafiltered using an 1-ft.sup.2 30 kDa cutoff cellulosic Amicon wound spiral cartridge at an operating pressure of 20 psi.

The final concentrate (ca. 400 mls, 6-7 mmhos/cm) was passed through a 0.2.mu. cellulose acetate filter. The filtered PA was loaded onto a Poros IIQ perfusion chromatography column using a quaternary Waters 600E HPLC pump. The column was prepared by hydrating seven grams of the Poros IIQ perfusion resin in twice the packed bed volume of 2% (w/v) NaCl. After settling the resin was resuspended in six times the packed bed volume of 25 mM DEA pH 8.9, 50 mM NaCl, 7.5% (v/v) ethylene glycol and allowed to settle overnight at room temperature. The resin was then resuspended in three times the packed bed volume and finally in one and one-half times the final volume before the slurry was extensively degassed using a vacuum pump (vacuum unknown). The entire degassed slurry was then transferred to a Waters AP 20.times.100 mm glass HPLC column and the column was packed in one step using the Waters 600E pumps at a flow rate of 20 mls/min and a backpressure of 650 psi at room temperature. The column separation efficiency was then tested at a flow rate of 10 mls/min using a linear 1 M NaCl gradient and ovalbumin 5 mg/ml (Sigma) and bovine serum albumin 10 mg/ml (Sigma) in DEA as buffer as standard proteins. Approximately 100 mls of PA (ca. 20-30 mg PA) cooled to 4-6.degree. C. was applied to the column and followed with a 20 min wash in the starting buffer at room temperature to elute unbound material. The column was then developed with a linear gradient to 30% of the 1 M NaCl DEA elution buffer. The purified PA was found to elute between 10-15%, while the smaller molecular weight proteolytic breakdown products eluted as a shoulder or partially resolved peak at 16-20% of the elution buffer. The resolution of the two peaks was found to be a function of content of PA proteolytic degradation products. The eluant was monitored at 280 nm and peak fractions were collected by manual triggering of an ISCO fraction collector. Samples of the peak fractions were diluted into 5-10 volumes of TRIS pH8.0, 0.1 mM PMSF, 50 AM OP, 1 mM EDTA buffer and concentrated using Amicon Centricon 30 concentrators at 4500.times.g at 4.degree. C. to approximately the initial sample volume. An equal volume of SDS-PAGE solubilization buffer was added to the sample immediately prior to heating at 95.degree. C. for 5 min. Purity was assessed from 8-25% SDS-PAGE PHAST gels (Pharmacia) and fractions with the highest purity were combined and dialyzed against 40-50 volumes of 25 mM DEA pH8.9, 50 mM NaCl, 0.1 mM PMSF and 2 mM EDTA at 4.degree. C. for at least 16 hrs. Fractions judged empirically to be less than 95% pure were rechromatographed under the

same conditions and purity of the fractions was reassessed as described above. All fractions of greater than 95% purity were ultimately combined, aliquoted and frozen at -70.degree. C. subsequent to determination of the total PA concentration.

Analysis and characterization of purified 83 kDa PA: Purified PA was quantitated by measuring UV-absorption at 280 nm using the relationship of 1 A.sub.280nm in a 1 cm pathlength cuvette is equals 1 mg PA/ml (Leppla, 1988). Results obtained in this manner were confirmed using the Bio-Rad Bradford protein assay under conditions suggested by the manufacturer. PA purity was assessed using SDS-PAGE under conditions described above. Capillary electrophoresis analytical assays have also proven promising in the assessment of PA purity and amounts of residual protease inhibitors in final product. Feasibility studies using a 47 cm.times.50 pm uncoated silica capillary and borate/SDS/acetonitrile buffer revealed an excellent separation of the protein from residual protease inhibitors. Quantitation of both protein and inhibitors has also proven possible, but the technique remains limited by the relatively high limits of detection (1 mM EDTA, 0.1 mM PMSF, and 0.05 mM OP) under current conditions. Automated N-terminal sequencing was carried out with purified PA using an Applied Biosystems 470A sequenator after desalting over Bio-Rad PD10 columns equilibrated with 5 mM NaCl and 1 mM CaCl.sub.2. A unique N-terminal sequence was found and the first six residues of the sequence were identical to PA from the endogenous plasmid pXO1 harbored by the USAMRIID B. anthracis Sterne strain. In addition, the sequence corresponded exactly with the published DNA derived protein sequence (Welkos et al.). Native gel electrophoresis under non-denaturing conditions revealed that PA purified from .DELTA.Sterne-1(pPA102)CR4 also exhibited the microheterogeneity noted previously for PA produced by the Sterne strain. Cytotoxicity assays of the product using the macrophage lysis assay (Friedlander et al.) revealed that the titration curve of biological activity for PA from .DELTA.Sterne-1 (pPA102)CR4 was indistinguishable from that generated for PA from the Sterne strain.

Evaluation of .DELTA.Sterne-1(pPA102)CR4:

EXAMPLE 1

B. Anthracis .DELTA.Sterne-1(pPA102)CR4 was compared with its parent spore-forming strain B. anthracis .DELTA.Sterne-1(pPA102). Both organisms were plated onto sheep blood agar (a preferred medium for promoting bacterial spore production) and grown at 37.degree. C. for 1 day, after which the temperature was lowered to 25.degree. C. for 4 days. The two strains were also grown in liquid Leighton-Doi medium, which is designed to promote spore production, for 1 day at 37.degree. C. followed by 4 days growth at 25.degree. C. Growth from both agar and broth cultures were examined under phase contrast microscopy for the presence of spores. Growth from all four cultures were then resuspended in phosphate buffered saline to a concentration of about 10.sup.9 colony-forming units (CFU) per ml. All four cultures were then heat shocked at 64.degree. C. for 60 minutes to kill vegetative cells. Aliquots of 0.1 ml of the heat shocked material was then plated out onto sheep blood agar and incubated at 37.degree. C. for 2 days.

Results:

B. anthracis .DELTA.Sterne-1(pPA102): Spores were seen under microscopic examination of material from both the sheep blood agar cultures and the Leighton-Doi medium cultures. On sheep blood agar plates containing heat shocked culture material from both sheep blood agar cultures and Leighton-Doi medium cultures, there was confluent growth. The data clearly indicate that B. anthracis .DELTA.Sterne-1(pPA102) forms spores.

B. anthracis .DELTA.Sterne-1(pPA102) CR4: No spores were seen under microscopic examination of material from both the sheep blood agar cultures and the Leighton-Doi medium cultures. On sheep

blood agar plates containing heat shocked cultures, there was no growth whatsoever. The data clearly indicate the B. anthracis .DELTA.Sterne-1(PPA102) CR4, which has been deposited in the American Type Culture Collection and has been assigned ATCC designation 69714, does not form spores. The deposit at the American Type Culture Collection located at 12301 Parklawn Drive, Rockville, Md. 20852, USA was made on Nov. 16, 1994.

EXAMPLE 2

B. anthracis .DELTA.Sterne-1(pPA102)CR4 was grown in an FA medium fermentor culture. No spores were seen upon phase contract microscopic examination. Only medium-length and long chains of bacilli were seen. Dilution plate counts on the culture determined that the culture contained 1.86.times.10.sup.9 CFU per ml. Three ml of culture was heat shocked at 60.degree. C. for 60 minutes, then 0.2 ml was plated onto each of 5 plates of Tryptic soy agar. After incubation for 2 days at 37.degree. C., no colonies were seen on the agar plates, indicating that spore production in the fermentor was less than 1 per 1.86.times.10.sup.9 CFU. On two other fermentation runs with this strain, similar results were obtained. No revertants to the parent spore-forming phenotype were observed.

The above process using an FA medium fermentor culture was repeated using the parent strain B. anthracis .DELTA.Sterne-1(pPA102). Growth on the tryptic soy agar after heat shock resulted in a total of 1000 total colonies, indicating that the parent strain B. anthracis .DELTA.Sterne-1(pPA102) had about 1000 spores per ml in the FA medium, or 1 spore per 106 CFU in the non-heat shocked medium.

EXAMPLE 3

Protective antigen (PA) was prepared in accord with the teachings under Materials and Methods as described above. The purified PA of B. anthracis .DELTA.Stern-1(pPA102)CR4 was mixed in different buffers (phosphate buffered saline, HEPES, Tris, glycyl glycine (GG), sodium citrate, for example) and combined with monophosphoryl lipid A (MPL), Squalene, Tween 80 and lecithin. The mixture was then lyophilized. At 0 and 4 weeks, vials of lyophilized MPL/PA/emulsion were reconstituted in phosphate buffered saline (PBS) and injected in 0.5 ml doses containing 50 .mu.g of PA per dose. At 10 weeks, the guinea pigs were aerosol challenged with approximately 36 medial lethal doses of virulent Bacillus anthracis spores of the Ames strain. The following data shows status two weeks after the challenge.

Vaccine	S/T*	%	Anti-PA**
PA in PBS (+ MPL emulsion)	10/12	83	29,427
PA in GG (+ MPL emulsion)	14/16	88	23,713
PA in Tris (+ MPL emulsion)	15/16	94	27,384
PA in HEPES (+ MPL emulsion)	15/15	100	25,482
PA in Citrate (+ MPL emulsion)	16/16	100	31,622
PBS	0/4	0	<10

*Survived/Total, day 14 post-challenge

**Prechallenge serum titers to PA were determined by enzyme linked immunosorbent assay. The geometric mean reciprocal titers were calculated for each group and are expressed in this table.

* * * * *

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United States Patent **6,316,006**
Worsham , et al. **November 13, 2001**

Asporogenic B anthracis expression system

Abstract

This invention relates to a bacterial expression system for production of protective antigen (PA) against bacillus anthracis. Recombinant asporogenic B. anthracits that are derived from .DELTA.Sterne-1 (pPA102) and show inability to bind the dye when grown on Congo Red Agar can be screened and asporogenic strains isolated using methods of the invention. organisms of the invention lacking spore-forming function may be killed by heat shock at temperatures as low as 60.degree. C. for 60 minutes. Hence, contamination of the environment with viable spore-forming organisms is easily avoided and decontamination is easily accomplished.

Inventors: **Worsham; Patricia** (Jefferson, MD); **Friedlander; Arthur M.** (Gaithersburg, MD); **Ivins; Bruce** (Frederick, MD)

Assignee: **The United States of America as represented by the Secretary of the Army** (Washington, DC)

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Ivins et al. Infect. Immun. Nov. 1986. 54(2): 537-542.*
Ivins et al. Infect. Immun. May 1986. 52(2): 454-457.

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Claims

What is claimed is:

1. A recombinant asporogenic B. anthracis isolated from .DELTA.Sterne-1 (pPA102) which shows inability to bind the dye when grown on Congo Red Agar.

2. A B. anthracis of claim 1 which is B. anthracis .DELTA.Sterne-1(pPA102)CR4.

3. A composition comprising the organism of claim 1 in a growth medium.

4. A composition comprising the organism of claim 2 in a growth medium.

Description

FIELD OF THE INVENTION

This invention relates to the bacterial expression system, production and use of protective antigen (PA) against Bacillus anthracis. The PA immunogen is useful in vaccine against human anthrax. The PA can be produced by an asporogenic organism which overproduces the desired antigen, which is then harvested from the supernatant.

BACKGROUND OF THE INVENTION

Bacillus anthracis is the etiologic agent responsible for anthrax, a disease often found in persons exposed to infected animals or their products. Persons particularly exposed to animals include veterinarians, laboratory technicians, ranchers and employees working with skin or hair of animals. The mode of entry into the body may be the skin or, when contaminated meat is eaten, the gastrointestinal tract. Inhaling of spores can cause inhalation anthrax, a disease that can be fatal. Vaccines against Bacillus anthracis have been available. Virulent strains of the organism produce two toxins and a poly-D-glutamic acid capsule which are coded for on two endogenous plasmids, pX01 and pX02, respectively. Loss of either of the plasmids results in an attenuated strain of reduced virulence, while loss of both results in an avirulent organism. The history of the USAMRIID Sterne strain of B. anthracis prior to 1981 is uncertain, though it is believed to be derived from the Sterne strain isolated at the Onderstpoort Research Laboratory in Pretoria, South Africa.

In 1985 the Bacillus anthracis protective antigen (PA) gene was cloned into a plasmid (pUB110) resulting in the formation of a recombinant plasmid identified as pPA102, which was reported in the literature (***Ivins*** and Welkos, Infection and Immunity, S54:537-542 (1986)). The production of vaccines lacking lethal factor was possible thereby. However, a primary problem remained, since the Bacillus anthracis formed spores. Once spores have formed, they persist in the environment for months and years. Once the laboratory environment contains such spores, it is very difficult to free the environment

of the spores.

It was also previously reported that protective antigen (PA) could be produced in baculovirus. [Iacono-Connors, et al., Infection and Immunity, 58:366-372 (1990); Iacono-Connors, et al., Infection and Immunity, 59:1961-1965 (1991)] A major problem in production of the PA in the baculovirus disclosed therein is that the desired antigen requires a complex purification process. Even after purification by immuno-affinity chromatography, undesired cellular material continues to contaminate the desired product.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention provides organisms which produce protective antigen (PA) lacking lethal factor and edema factor proteins which, when present as contaminants in vaccine, can cause serious side effects. The producing organisms of the invention are also, surprisingly, non-sporulating. Furthermore, the desired antigen is expressed into the supernatant. Hence, the protective antigen produced is easily purified and, though protective, does not cause many of the troublesome side effects of prior art vaccines. The organisms of the invention lacking spore-forming function may be killed by heat shock at temperatures as low as 60.degree. C. for 60 minutes. Hence, contamination of the environment with viable spore-forming organisms is easily avoided and decontamination is easily accomplished.

Genesis of .DELTA.Sterne-1(pPA102)CR4:

A 6 kb Bam HI fragment harboring the PA structural gene isolated from the endogenous Sterne plasmid pX01 was ligated into plasmid pBR322 and cloned into Escherichia coli bacteria (Vodkin and Leppla, 1983). From the resultant recombinant plasmid pSE36, the 6kb fragment was then subcloned into the gram-positive vector PUB110 using the Bam HI restriction site. The resulting plasmid was transformed into B. subtilis IS53 and two stable PA producing, kanamycin resistant isolates were found (pPA101 and pPA102) (***Ivins*** and Welkos, 1986). Subsequent analysis of the plasmids revealed that both had suffered spontaneous deletions. The pPA102 was found to have lost 4.2 kb of DNA from 363 bp 3' of the kanamycin resistance gene to approximately 164 bp 5' of the start of the PA structural gene, a result consistent with the observed inactivation of the phleomycin resistance gene of pUB110. The plasmid was then electrotransformed into .DELTA.Sterne-1, a plasmid-free strain of B. anthracis (Infection and Immunity, 52:454-458 (1986) and transformants were selected for kanamycin resistance. Transformants displaying a stable PA+, kanamycin resistant, (LF-, EF-, capsule-) phenotype were selected. This strain, .DELTA.Sterne-1(pPA102), was then subjected to Congo Red agar selection for mutants displaying an inability to bind the dye, a characteristic known to correlate with an asporogenic phenotype (Worsham, submitted). The selected isolate, now designated .DELTA.Sterne-1(pPA102)CR4 was further subcultured three times to insure that a single clone was isolated. This clone has served as the seed stock for all research and development of fermentation conditions, and purification of PA.

Materials and Methods:

Fermentation Conditions

Media: FA medium was used for all plates and liquid cultures described here unless otherwise specified. FA medium consisted of 33 g/l tryptone (Difco), 20 g/l yeast extract (Difco), 2 g/l L-histidine, 8 g/l Na2HPO4, 7.4 g/l NaCl, 4 g/l KH2PO4 adjusted to pH 7.4 with NaOH.

Precultures: A working stock of .DELTA.Sterne-1(pPA102)CR4 was prepared from the seed culture by streaking cells on an FA medium plate containing 40 .mu.g/ml of kanamycin. A sweep from the confluent growth zone on plate was cultured one time in liquid FA medium supplemented with

kanamycin 40 .mu.g/ml to a final O.D..sub.600nm of 4.0. This culture was checked for purity by streaking on SBA plates, and diluted into multiple vials containing sterile 100% glycerol to a final glycerol concentration of 50% (V/V). These stocks were stored at -70.degree. C. A single vial was removed at the start of each fermentation cycle and discarded after use. The defrosted cells were streaked onto FA plates containing 40 .mu.g/ml kanamycin and incubated at least 16 hrs at 37.degree. C. After 16 hrs the plated cells were used to inoculate 50 mls of FA medium supplemented with 40 .mu.g/ml kanamycin in a 250 ml baffled-Erlenmeyer flask (Bellco Laboratories). The culture was incubated at 370.degree. C. at 200 rpm for 6 hrs or until an O.D..sub.600nm of 4-6 was obtained. The cells were then subcultured into 50 mls of FA medium in an identical flask under identical conditions. After 6 hrs, or a culture O.D..sub.600nm of 6.2-6.5, a 1.6% (v/v) inoculum was transferred to 300 mls of FA medium supplemented with 40 .mu.g/ml kanamycin in a 2 liter baffled Erlenmeyer and incubated at 37.degree. C. at 200 rpm for 7hrs, or until a final O.D..sub.600nm of 3.5-3.7 was achieved.

Fermentation conditions: The fermentations described here were carried out using a New Brunswick Bio-Flo 3000 equipped with a 5.0 liter working volume glass vessel and stainless steel headplate and hemispherical bottom cooling dish. Four liters of FA medium were added to the vessel, which had been previously completely disassembled, scrubbed in a dilute Envirochem solution and autoclaved for 15 min after the addition of 4 liters of H.sub.2 O. The polarographic DO.sub.2 probe (Ingold) and pH probes (either liquid or gel filled, Ingold) were also inserted and all addition and sampling ports were sealed or clamped and wrapped in aluminum foil. Addition lines consisted of surgical grade autoclavable Tygon tubing (Thomas Scientific) and all lines were sealed with the exception of the condenser, which was left open to permit pressure release, but covered with aluminum foil. The vessel was autoclaved using a 10 min exposure time at 121.degree. C. and removed from the autoclave as soon as sufficient cooling had occurred to allow opening of the autoclave. The vessel was then immediately connected to the fermentor unit and the condenser line was connected to a sterile liquid trap and 0.2 .mu. capsule filter to avoid the introduction of contaminants during the cooling process. The vessel was then cooled to 37.degree. C. using the fermentor driven temperature control and positive pressure was provided using compressed sterile filtered air. Once the vessel had cooled to 37.degree. C. sterile filtered kanamycin was added to a final concentration of 40 .mu.g/ml. The agitation was activated at 150 rpm and aeration was adjusted to 1-1.2 volume/volume/min (vvm) and antifoam C (DOW), that had been diluted 10-fold into H.sub.2 O and autoclaved, was added to a final concentration of 200 ppm.

A preinoculation sterility check was conducted for a minimumi of 16 hrs during which time pH, agitation and temperature were continually monitored. After the 16 hrs required for DO.sub.2 probe polarization, the DO.sub.2 was also monitored along with turbidity. The DO.sub.2 probe was calibrated using an INGOLD calibration device which sets the zero value to 4 mA and 100% to the oxygen tension determined by the solubility of oxygen in the medium after aeration and agitation at 37.degree. C. The calibration and response of the electrode was then checked by sparging with pure N.sub.2. The vessel was judged to be sterile if the pH and DO.sub.2 remained constant and no increase in turbidity was observed. It should be emphasized that the short autoclave cycle for vessel sterilization was required to minimize caramelization, Millard and other chemical degradation reactions which are problematic due to the high concentrations of yeast extract and tryptone in FA medium. As an additional confirmation of sterility, 50 mls was aseptically removed from the fermentor to a 250 mls Erlenmeyer and incubated at 37.degree. C. at 200 rpm for 48 hrs with no sign of growth. Under the conditions outlined here contamination has not been observed in more than 10 fermentation cycles.

Once the sterility of the vessel had been verified, the 300 ml inoculum described above was added to the vessel through the addition port of the headplate and the initial O.D..sub.600nm was recorded. A sample of the inoculum was also streaked on SBA plates and incubated for 48 hrs at 37.degree. C. to verify inoculum purity. Using the Bio-Flo 3000, aeration was maintained at 75% of saturation by increasing agitation from the initial 150 rpm to a maximum of 400 rpm and ultimately by supplementing the 1 vvm

aeration rate with pure oxygen. The mixture rate and percentages of air and oxygen were controlled by a solenoid and algorithm developed by New Brunswick Scientific. Both gases had a working pressure of approximately 10 psi.

The O.D..sub.600nm dry cell weight (DCW), production of PA, DO.sub.2, pH, agitation and temperature were monitored throughout each fermentation cycle. The O.D..sub.600nm DCW and PA production analysis were carried out by manually sampling the fermentation liquor at hourly intervals using a sterile sampling port. O.D..sub.600nm was measured after dilution of the culture using sterile medium prepared for that fermentation. For each O.D..sub.600nm determination, two appropriate dilutions were made and results were considered acceptable only when both dilutions yielded a linear response. DCWs were determined starting with a 2 hr point by centrifuging 10 mls of fermentation liquor at 11,953 .times.g for 10 min, resuspending the cell pellet in 10 mls of sterile PBS and pelleting the cells again under the same conditions. The cell pellet was resuspended in a minimal volume of PBS and transferred quantitatively to a preweighted Eppendorf centrifuge tube and centrifuged at 14,000 rpm for 5 min. Excess PBS was removed and the cell pellet was dried in a speed-vac for 72 hrs under vacuum and a medium heat setting. A final analysis of the dry weight versus O.D..sub.600nm revealed that the relationship between the two parameters was adequately fit with a linear function.

Fermentation Reproducibility: The reproducibility of the cell growth parameters, biomass and PA production in fermentations carried out with the Bio-Flo 3000 under the conditions described above have been summarized in Table I below. Two fermentations were carried out at 75% of the maximum dissolved oxygen concentration in a strict batch mode with no pH control or additions other than antifoam C. The variation in the agitation rate during the first 100 min of the fermentation was the result of the AGDO.sub.2 (agitation DO.sub.2) control mode chosen to maintain the dissolved oxygen tension at 75% of the maximum. Briefly, this algorithm attempts to control the oxygen tension by first altering the agitation rate until this proves insufficient, at which point the process air is supplemented with pure oxygen as needed to maintain the desired DO.sub.2. The temperature was held constant at 37.degree.+/-0.1.degree. C. The pH was monitored, but not regulated as an internal check on the aeration of the vessel during the course of the fermentation. The fact that the pH revealed a decrease on only 0.2 pH units in the first 150 min was consistent with an aerobic culture metabolizing the limited carbohydrate supplied with the yeast extract to CO.sub.2 and organic acids. Once the carbohydrate was exhausted after ca. 150 min, the bacillus switched to the utilization of amino acids and peptides for a carbon source, which under aerobic conditions resulted in the release of NH.sub.4 OH and the observed increased culture pH.

These fermentations were sampled on an hourly basis and allowed to proceed until no further increase in O.D..sub.600nm was observed over two time points. O.D..sub.600nm , DCW analysis and product measurements were carried out for each sample as described above. Samples for PA production were sterile filtered followed by the addition of HEPES and the complete protease cocktail as described under PA quantitation. The samples were concentrated, desalted and ultimately concentrated 80-fold prior to being analyzed using SDS-PAGE. The major band of the gel corresponded to the 83 kDa PA product. An increasing in the intensity of the protein band was seen with increasing fermentation time. Study of a Western blot of another time course of a batch fermentation was developed with polyclonal rabbit anti-PA83. Comparison revealed that along with increasing PA 83 kDa there was also a pronounced increase in the abundance and form of proteolytic degradation products of PA.

TABLE 1

Summary of Aerobic .DELTA.Sterne-1(pPA102)CR4 Fermentations

Fermentation Conditions	Final Conc. (.mu.g PA83/ml	Final Yield (mg PA83)	Final Yield (mg PA83/g DCW)	Specific Growth Rate	Doubling Time T.sub.D (min)
Aerobic, Batch	51	235	8.10	0.0132	

min.sup.-1	53				
Aerobic, Batch		64	301	10.7	0.0136
min.sup.-1	51				
Aerobic, Batch		45	225	7.40	0.0136
min.sup.-1	51				
pH constant					
Aerobic, Fed-Batch		68	360	ND	0.0116
min.sup.-1	60				
(noncontinuous)					
DCW = dry cell weight					

The data presented in Table 1 demonstrated that the PA yield on a unit volume and biomass basis, as well as the cell growth parameters, were reproducible for the batch fermentations conducted without pH control. The final fermentation pH values of 8.57 and 8.67 after an elapsed fermentation time of ca. 8 hrs were also comparable. The effect of prolonged exposure to these mildly alkaline conditions on cell growth, PA production and subsequent degradation was investigated by repeating the fermentation at a constant pH of 7.50+/-0.05 pH units. This was accomplished using the immersed vessel pH probe and automated additions of 2 N HCl or 1 N NaOH. The results shown in Table 1 demonstrate that there was no clear effect of constant pH on any of the parameters evaluated. SDS-PAGE analysis of the fermentation time points sampled for PA production also revealed no significant differences.

The final fermentation presented in Table 1 was a noncontinuous fed-batch trial during which 1/10 volume of a 10-fold concentrate of sterile-filtered tryptone was added after 5 hrs or an O.D..sub.600nm of 7.5. The result suggested that such fed-batch fermentations provide possible protocols for improvement to increase yield and decrease proteolysis.

Harvest conditions: Fermentations were allowed to proceed until no further increase in O.D..sub.600nm was observed. At this point, the fermentor was cooled to 10.degree. C. and the protease inhibitors phenylmethylsulfonyl fluoride (PMSF), 1,10-phenanthroline (OP) and ethylenediamine tetraacetate (EDTA) were added to final concentrations of 0.1, 0.05 and 2 mM, respectively. The cells were then pumped from the fermentor vessel at room temperature using an Amicon DC10L concentrator equipped with a 10-ft.sup.2 0.1 .mu. polysulfone hollow-fiber cartridge. The fermentor liquor was diluted 1:1 with 25 mM diethanolamine (DEA), 50 mM NaCl, 2 mM EDTA, 0.1 mM PMSF adjusted to pH 8.9 with HCl. The filtrate was collected at an operating pressure of less than 20 psi and transferred directly to a second Amicon DC10L equipped with two 30 kDa cutoff 10-ft.sup.2 wound spiral cellulosic cartridges. The filtrate was concentrated approximately 10-fold before being subjected to diafiltration at an operating pressure of less than 30 psi against the same buffer. The conductivity of the retentate was monitored with an Amber Sciences conductivity meter and platinum immersion pencil-type electrode. The diafiltration step generally required 20 liters of buffer, but was considered complete only after the conductivity of the concentrated retentate was equivalent to that of the starting buffer.

Quantitation of 83 kDa PA in crude fermentation liquor: The fermentation liquor was sampled using a sterile port at regular intervals throughout the fermentation process. The samples for PA determination were filtered through syringe type 0.2 .mu. cellulose acetate filters, 0.1 mM PMSF, 2 mM EDTA, 50 .mu.M OP and 20 mM HEPES pH7.3 were added and the samples were frozen at -70.degree. C. The samples were defrosted on ice and concentrated using Amicon Centricon 30 concentrators at 4500 .times.g. The samples were concentrated approximately 10-fold, diluted to the original volume with 10 mM TRIS pH8.0, 0.1 mM PMSF, 2 mM EDTA, 0.05 .mu.M OP and concentrated again. The concentrated sample was desalted again using the same buffer, frozen and finally lyophilized using a Speed-Vac. The dried samples were dissolved in 25 .mu.l of the TRIS buffer described above and

diluted 1:1 with a 2.times.SDS solubilization buffer consisting of 50 mM Na.sub.2 CO.sub.3, 4% (w/v) SDS, 12% (v/v) glycerol, 2% (v/v) 2-mer-captoethanol and 0.01% (w/v) Bromphenol Blue prior to heating at 95.degree. C. for 5 min. The fermentation samples containing varying amounts of PA 83 kDa were solubilized as described above and run on a Daiichi 4-20% gradient TRIS/TRICINE gel to approximate total yield of PA. Two hundred to 2000 ng samples of purified PA were solubilized in the same buffer and loaded onto the gel in constant total volume of 3 .mu.l. Three or four appropriate dilutions of the fermentation samples determined from the first gel were loaded onto the gel with the standards and electrophoresed at 100 V initially and 140 V once the samples entered the separating gel and until the Bromphenol Blue dye reached the bottom edge of the separating gel. The gel was then fixed in 10% (v/v) acetic acid 20% (v/v) MeOH for 10 min, rinsed with MQ H.sub.2 O and stained with Coomassie Brilliant Blue 0.05% (w/v) in 10% (v/v) acetic acid for a minimum of 16 hrs to allow complete and uniform staining. The stained gel was then destained in 10% (v/v) acetic acid until the background contained no visible residual dye. The gel was then scanned on a laser densitometer (LKB, Ultrascan XL Laser Densitometer). Representative portions of the gel without protein were randomly chosen and scanned to determine background absorption for an accurate baseline. The region to be scanned for each lane containing PA was then visually aligned to insure that the entire protein peak and adequate baseline were included in each scan. The scans were completed and the integration values were determined using the LKB preprogrammed Gaussian algorithm and later were confirmed by cutting out individual peaks and manually integrating based on peak weight. The resulting integration values were plotted using Sigmaplot (Jandel). Linear regression of the results revealed typical r values of 0.992-0.996. The linear standard curve was then used to quantitate the amount of 83 kDa PA in the various fermentation samples based on the same integration methods.

Purification: The exact volume and conductivity of the PA in DEA buffer was determined and solid KCl was added to the solution to a final concentration of 30 mM and conductivity of 10-11 mmhos/cm. The PA was pumped with a peristaltic pump through a monoQ column prepared by collecting 100 mls of hydrated Bio-Rad Macro Prep 50Q on a sintered glass filter and washing sequentially with 1 liter of 25 mM DEA, 50 mM NaCl, 1 mM EDTA, 50 .mu.M OP and 0.1 mM PMSF pH8.9 and 1 liter of the same buffer with 30 mM KCl added. The conductivity (10-11 mmhos/cm) and pH of 8.9 of the eluate from the Macro Prep 50Q after the second wash were comparable to that of the PA solution after addition of KCl. The Macro Prep 50Q resin was then degassed and slurry packed into a Pharmacia K column with a Rainin Rabbit-Plus peristaltic pump at 48 rpm and a flow rate of 15 mls/min. The final column volume was (5.times.5 cm) 98 mls. The PA solution was pumped through the Macro Prep 50Q column at a rate of 10 mls/min and the eluate was collected until all of the PA sample volume was loaded and the column washed with an additional 100 mls of DEA/KCl buffer. The eluate containing unbound PA was concentrated and diafiltered using an 1-ft.sup.2 30 kDa cutoff cellulosic-Amicon wound spiral cartridge at an operating pressure of 20 psi. The final concentrate (ca. 400 mls, 6-7 mmhos/cm) was passed through a 0.2 A cellulose acetate filter. The filtered PA was loaded onto a Poros IIQ perfusion chromatography column using a quaternary Waters 600E HPLC pump. The column was prepared by hydrating seven grams of the Poros IIQ perfusion resin in twice the packed bed volume of 2% (w/v) NaCl. After settling the resin was resuspended in six times the packed bed volume of 25 mM DEA pH 8.9, 50 mM NaCl, 7.5%(v/v) ethylene glycol and allowed to settle overnight at room temperature. The resin was then resuspended in three times the packed bed volume and finally in one and one-half times the final volume before the slurry was extensively degassed using a vacuum pump (vacuum unknown). The entire degassed slurry was then transferred to a Waters AP 20.times.100 mm glass HPLC column and the column was packed in one step using the Waters 600E pumps at a flow rate of 20 mls/min and a backpressure of 650 psi at room temperature. The column separation efficiency was then tested at a flow rate of 10 mls/min using a linear 1 M NaCl gradient and ovalbumin 5 mg/ml (Sigma) and bovine serum albumin 10 mg/ml (Sigma) in DEA as buffer as standard proteins. Approximately 100 mls of PA (ca. 20-30 mg PA) cooled to 4-6.degree. C. was applied to the column and followed with a 20 min wash in the starting buffer at room temperature to elute unbound material. The column was then developed with

a linear gradient to 30% of the 1 M NaCl DEA elution buffer. The purified PA was found to elute between 10-15%, while the smaller molecular weight proteolytic breakdown products eluted as a shoulder or partially resolved peak at 16-20% of the elution buffer. The resolution of the two peaks was found to be a function of content of PA proteolytic degradation products. The eluant was monitored at 280 nm and peak fractions were collected by manual triggering of an ISCO fraction collector. Samples of the peak fractions were diluted into 5-10 volumes of TRIS pH8.0, 0.1 mM PMSF, 50 .mu.M OP, 1 mM EDTA buffer and concentrated using Amicon Centricon 30 concentrators at 4500.times.g at 4.degree. C. to approximately the initial sample volume. An equal volume of SDS-PAGE solubilization buffer was added to the sample immediately prior to heating at 95.degree. C. for 5 min. Purity was assessed from 8-25% SDS-PAGE PHAST gels (Pharmacia) and fractions with the highest purity were combined and dialyzed against 40-50 volumes of 25 mM DEA pH8.9, 50 mM NaCl, 0.1 mM PMSF and 2 mM EDTA at 4.degree. C. for at least 16 hrs. Fractions judged empirically to be less than 95% pure were rechromatographed under the same conditions and purity of the fractions was reassessed as described above. All fractions of greater than 95% purity were ultimately combined, aliquoted and frozen at -70.degree. C. subsequent to determination of the total PA concentration.

Analysis and characterization of purified 83 kDa PA: Purified PA was quantitated by measuring UV-absorption at 280 nm using the relationship of 1 A.sub.280nm in a 1 cm pathlength cuvette is equals 1 mg PA/ml (Leppla, 1988). Results obtained in this manner were confirmed using the Bio-Rad Bradford protein assay under conditions suggested by the manufacturer. PA purity was assessed using SDS-PAGE under conditions described above. Capillary electrophoresis analytical assays have also proven promising in the assessment of PA purity and amounts of residual protease inhibitors in final product. Feasibility studies using a 47 cm.times.50 .mu.m uncoated silica capillary and borate/SDS/acetonitrile buffer revealed an excellent separation of the protein from residual protease inhibitors. Quantitation of both protein and inhibitors has also proven possible, but the technique remains limited by the relatively high limits of detection (1 mM EDTA, 0.1 mM PMSF, and 0.05 mM OP) under current conditions. Automated N-terminal sequencing was carried out with purified PA using an Applied Biosystems 470A sequenator after desalting over Bio-Rad PD10 columns equilibrated with 5 mM NaCl and 1 mM CaCl.sub.2. A unique N-terminal sequence was found and the first six residues of the sequence were identical to PA from the endogenous plasmid pX01 harbored by the USAMRIID B. anthracis Sterne strain. In addition, the sequence corresponded exactly with the published DNA derived protein sequence (Welkos et al.). Native gel electrophoresis under non-denaturing conditions revealed that PA purified from .DELTA.Sterne-1(pPA102)CR4 also exhibited the microheterogeneity noted previously for PA produced by the Sterne strain. Cytotoxicity assays of the product using the macrophage lysis assay (Friedlander et al.) revealed that the titration curve of biological activity for PA from .DELTA.Sterne-1 (pPA102)CR4 was indistinguishable from that generated for PA from the Sterne strain.

Evaluation of .DELTA.Sterne-1(pPA102)CR4:

EXAMPLE 1

B. Anthracis .DELTA.Sterne-1(pPA102)CR4 was compared with its parent spore-forming strain B. anthracis .DELTA.Sterne-1(pPA102). Both organisms were plated onto sheep blood agar (a preferred medium for promoting bacterial spore production) and grown at 37.degree. C. for 1 day, after which the temperature was lowered to 25.degree. C. for 4 days. The two strains were also grown in liquid Leighton-Doi medium, which is designed to promote spore production, for 1 day at 37.degree. C. followed by 4 days growth at 25.degree. C. Growth from both agar and broth cultures were examined under phase contrast microscopy for the presence of spores. Growth from all four cultures were then resuspended in phosphate buffered saline to a concentration of about 10.sup.9 colony forming units (CFU) per ml. All four cultures were then heat shocked at 64.degree. C. for 60 minutes to kill vegetative cells. Aliquots of 0.1 ml of the heat shocked material was then plated out onto sheep blood agar and

incubated at 37.degree. C. for 2 days.

Results:

B. anthracis .DELTA.Sterne-1(pPA102): Spores were seen under microscopic examination of material from both the sheep blood agar cultures and the Leighton-Doi medium cultures. On sheep blood agar plates containing heat shocked culture material from both sheep blood agar cultures and Leighton-Doi medium cultures, there was confluent growth. The data clearly indicate that B. anthracis .DELTA.Sterne-1(pPA102) forms spores.

B. anthracis A8tern-l(pPA102)CR4: No spores were seen under microscopic examination of material from both the sheep blood agar cultures and the Leighton-Doi medium cultures. On sheep blood agar plates containing heat shocked cultures, there was no growth whatsoever. The data clearly indicate the B. anthracis .DELTA.Sterne-1(pPA102)CR4, which has been deposited in the American Type Culture Collection and has been assigned ATCC designation 69714, does not form spores.

EXAMPLE 2

B. anthracis .DELTA.Sterne-1(pPA102)CR4 was grown in an FA medium fermentor culture. No spores were seen upon phase contract microscopic examination. Only medium-length and long chains of bacilli were seen. Dilution plate counts on the culture determined that the culture contained 1.86.times.10.sup.9 CFU per ml. Three ml of culture was heat shocked at 60.degree. C. for 60 minutes, then 0.2 ml was plated onto each of 5 plates of Tryptic soy agar. After incubation for 2 days at 37.degree. C., no colonies were seen on the agar plates, indicating that spore production in the fermentor was less than 1 per 1.86.times.10.sup.9 CFU. On two other fermentation runs with this strain, similar results were obtained. No revertants to the parent spore-forming phenotype were observed.

The above process using an FA medium fermentor culture was repeated using the parent strain B. anthracis .DELTA.Sterne-1(pPA102).

Growth on the tryptic soy agar after heat shock resulted in a total of 1000 total colonies, indicating that the parent strain B. anthracis .DELTA.Sterne-1(pPA102) had about 1000 spores per ml in the FA medium, or 1 spore per 10.sup.6 CFU in the non-heat shocked medium.

EXAMPLE 3

Protective antigen (PA) was prepared in accord with the teachings under Materials and Methods as described above. The purified PA of B. anthracis .DELTA.Stern-1 (pPA102)CR4 was mixed in different buffers (phosphate buffered saline, HEPES, Tris, glycyl glycine (GG), sodium citrate, for example) and combined with monophosphoryl lipid A (MPL), Squalene, Tween 80 and lecithin. The mixture was then lyophilized. At 0 and 4 weeks, vials of lyophilized MPL/PA/emulsion were reconstituted in phosphate buffered saline (PBS) and injected in 0.5 ml doses containing 50 .mu.g of PA per dose. At 10 weeks, the guinea pigs were aerosol challenged with approximately 36 medial lethal doses of virulent Bacillus anthracis spores of the Ames strain. The following data shows status two weeks after the challenge.

Vaccine	S/T*	%	Anti-PA**
PA in PBS (+ MPL emulsion)	10/12	83	29,427
PA in GG (+ MPL emulsion)	14/16	88	23,713
PA in Tris (+ MPL emulsion)	15/16	94	27,384
PA in HEPES (+ MPL emulsion)	15/15	100	25,482
PA in Citrate (+ MPL emulsion)	16/16	100	31,622
PBS	0/4	0	<10

*Survived/Total, day 14 post-challenge
**Prechallenge serum titers to PA were determined by enzyme linked immunosorbent assay. The geometric mean reciprocal titers were calculated for each group and are expressed in this table.

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The following investigation was conducted by Special Agent [redacted] between July 6 and 11, 2005:

[Administrative: During 2001, BRUCE EDWARDS IVINS used dial-up America Online (AOL) as his internet service provider (ISP). Investigation to date has revealed that based on IVINS' home telephone number, [redacted] the AOL software would have dialed three different numbers to connect to the internet. Two of the numbers [redacted] and [redacted] were leased to AOL by Genuity, Inc., and one was leased to AOL by UUNET, Inc. [redacted]

On July 6, 2005, writer contacted [redacted] UUNET, Inc., telephone number [redacted] in order to obtain Internet Protocol (IP) information on BRUCE EDWARDS IVINS for 2001. [redacted] advised that if IVINS connected to the number operated by UUNET to connect to AOL, they would have record of that connection. [redacted] further advised that she would query UUNET's databases to determine if IVINS' telephone number had ever dialed into UUNET's number.

On July 11, 2005, [redacted] contacted writer and advised that IVINS' home number had never been used to connect with UUNET.

[redacted] did not provide any additional information.

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DATE 12-15-2008 BY 60324 UC BAW/DK/TH

The following investigation was conducted by Special Agent on July 8, 2005:

During the review of electronic evidence in support of Major Case 184, draft agendas were found for the 4th International Conference on Anthrax, held in Annapolis, Maryland from June 10 thru 13, 2001. The conference was organized by scientists from the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), namely and BRUCE IVINS.

Evidence review revealed two drafts of the agenda, one labeled 4th Anthrax Wkshp.Scient.Prog#2 and the other labeled 4th Anthrax Wkshp.Sci.Prog#4 dated 6/9/00.

These agendas were compared to one another to determine any changes made between drafts. The results of the comparison is detailed below. Each noted difference will be labeled by the section where the difference is found. The agendas will be labeled Prog#2 and Prog#4. Copies of each agenda are attached to and made part of this document.

Header

Prog#4 is dated 6/9/00 while Prog#2 is not dated.

Prog#4 includes an additional "REF."labeled 4th Anthrax Wkshp.Lori#4.

Scientific Program section and "REF" section are in a different order on Prog#4 than on Prog#2.

Day 1: Opening Remarks

Prog#4 lists or (USAMRIID) as the first speaker while Prog#2 lists or (USAMRIID) as the first speaker.

Day 1: Section Two

Prog#4 header reads Anthrax: Threats and Risks while Prog#2 reads Risks and hazards.

Prog#4 chairman is or other CDC representative: or Rapid Response program POC etc.). Prog#2 chairman is or

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Day 1: Section Three

On Prog#4 under "Other methods for rapid detection), two additional items are listed than on Prog#2. These names are Diag. Sys. Div (USAMRIID); real-time PCR, Robotics and [redacted] (Tox, USAMRIID) - flow cytometry titer determination.

Day 2: Section Five

Prog#4 header reads "Cell Structure and Function" while Prog#2 reads "Structure and Function."

Day 2: Section Six

First subject in this section of Prog#4 reads "Plasmid and chromosomal regulation of toxin gene expression" while the first subjection of Prog#2 reads "The *pag* operon: Regulation of *PagA* by *PagR*"

"Updates" section of Prog#4 includes a topic titled "genetic constructs for improved expression of LF" while Prog#2 does not.

"Updates" section of Prog#2 includes a topic titled "New candidate vaccines" while Prog#4 does not.

"The interaction of *B. anthracis* with macrophages" section of Prog#4 includes topics titled "Macrophages in pathogenesis of anthrax" and Effect of LF on cytokine production by macrophages; [redacted] - USAMRIID) while Prog#2 does not. Prog#2 has a different title for this section, "The interaction of *B. anthracis* with macrophages in pathogenesis."

Day 3: Section Seven

Header on Prog#4 is labeled "Immunity and Vaccines" while Prog#2 is labeled "Vaccines and immune responses."

Prog#4 includes a presenter [redacted] under "Other experimental vaccines" while Prog#2 does not.

Prog#2 includes the verbiage "possible subjects.." after the topic titled "Correlates of immune protection" while Prog#4 does not.

Day 3: Section Eight

Prog#4 includes a line item titled "("PA32": [redacted] - Los Alamos) under "toxin competitive inhibitors" while Prog#2 does not.

Prog#4 assigns the section titled "Update on Antibiotics: efficacy, resistance (lectures or posters)" to [redacted] - USAMRIID while Prog#2 does not.

Additional Agenda

In addition to the abovementioned agendas, a preliminary agenda was written by [redacted] and sent to [redacted] BRUCE IVINS, [redacted] for input and feedback. Based on a comparison between the preliminary agenda and Prog#2, very little was changed. These same eight main topics were carried over from the preliminary agenda to Prog#2. A copy of the preliminary agenda is attached to and made part of this document.

Lead - [redacted]

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file: 4th Anthrax Wkshp.Sci.Prog#4 6/9/00

4th International Conference on Anthrax

Annapolis, MD U.S.A.

10 - 13 June 2001

ALL INFORMATION CONTAINED
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REF.: Files (1)4th Anthrax Wkshp reviewers (2) 4th Anthrax Wkshp.people
(3) 4th Anthrax Wkshp [redacted]4

Scientific Program [SUBJECT CATEGORIES and possible speakers and subjects]

DAY 1:

Opening Remarks (1) [redacted] (USAMRIID);
and? - (2) [redacted] [or present talk during the main "Conference Dinner": "emeritus speaker"]

#1: Ecology and Epidemiology
Chairman: [redacted]

Anthrax and the internet: 15 min.
A balance between immediacy and accuracy of information
[redacted]

2001 Global Anthrax Report 15 min.
[redacted]

Updates - Foci and outbreaks of human anthrax (possible lectures or posters)
Central Asian republics
Russia
U.S.A.: [redacted] or CDC rep [redacted]
China

Updates: Foci and outbreaks in animals (possible lectures or posters)
Canada/USA: domesticated and wild animals
[redacted]?
Australia: Update [redacted]
Africa: Updates
[redacted] -South Africa?

Late-breakers

#2: Anthrax: Threats and Risks
Chairman: [redacted] or other CDC representative:
[redacted] or Rapid Response program POC [redacted] etc.)

Bioterrorism and the alleged use of anthrax:
Overview and management

OR: Anthrax as a biological weapon:
Medical and Public Health Management
[Working Group for Civilian Biofense:

35 tapes, Phase II POIs
Ivins, Bruce, PST

Rep from JHU, DHHS/CDC, or USAMRIID (DOD - no?) [redacted] b6 b7C

Disposal of stockpiles in the FSU:
discovery of abandoned spore stockpiles in Uzbekistan/Kazahkstan
[A Russian - sensitivity issue?]

#3: Detection, Identification, and Classification of *B. anthracis*

Chairman: [redacted]

Genetic Diversity of *B. anthracis*

plasmid-specific diversity:
[redacted] (*pag* gene diversity)

chromosomal polymorphisms:
New markers and procedures
(MLVA/multiplex PCR)
vrr loci polymorphisms
[redacted]

Genetic markers for detection:
[redacted] RAPD fingerprinting)

Updates on other genetic markers (Lectures or Posters)
[redacted] et al.
[redacted] (SASP gene probes and Bacillus evolution)?

Immunological detection:
Antibody-based systems of detection in natural samples
[redacted] - NMRI

Monoclonal antibodies to spores and vegetative cells
[redacted]

Other methods for rapid detection (Lectures or Posters):
[redacted] NNRI); ribotyping
[redacted] (DARPA); molecular diagnostics and detectors
Diag. Sys. Div (USAMRIID); real-time PCR, robotics,
[redacted] (Tox, USAMRIID) - flow cytometry titer determination

Chairman's Overview: Identification and Diversity of *B. anthracis*

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DAY 1 or 2:

#4: Molecular Biology and Genomics
Chairman: [redacted]

The genome of *B. anthracis* strain Ames: sequence and analysis
[redacted] TIGR)

Sequence analyses of pX01 and pX02: Updates
[redacted] etc.)

Characerization of plasmid replication sequences
[redacted]

DAY 2:

#5: Cell Structure and Function:
Chairman: [redacted]
or [redacted]

Genetic Control of Sporulation and Germination OR
Genetc Control of Spore and Vegetative Cell Structure
(Review: [redacted]

Germination genes of *B. anthracis*:
(Ireland, germination loci)
[redacted] group, germination loci)

Characteristics of the exosporium
(Update: [redacted]

Spore Structural Proteins - [OR in Topic #3]
[redacted]

S-Layer Homologous Domains of bacterial surface proteins
[redacted] group [redacted]

#6: Genetic Regulation and Pathogenesis
Chairman: [redacted]

Plasmid and chromosomal regulation of toxin gene expression
[redacted]

Identification of receptor-binding regions in PA (two groups):
(1) [redacted] et al. [redacted]
(2) [redacted] et al. [redacted]

Oligomerization of PA and cellular uptake of toxin
[redacted]

Mechanism of anthrax toxin entry into cells
[redacted] (1) Characterization of the PA Channel
(2) A polarized epithelial cell model for toxin entry

Role of the Proteosome in LF toxicity
[redacted]et al.)

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Updates (lectures or posters)
LF Fusion -mediated delivery system, or
genetic constructs for improved expression of LF
[redacted] et al.)

LF Mechanism of Action (MAPKKs, etc.)
[redacted] or group (eg., [redacted]

The interaction of *B. anthracis* with macrophages:
Macrophages in pathogenesis of anthrax
[redacted]
Effect of LF on cytokine production by macrophages:
[redacted] - USAMRIID)

Anti-spore host responses?
[redacted]

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DAY 3:

Alternate: Combine categories #7 and #8: Immunoprophylaxis and Treatment
Chairmen: [redacted]

#7: Immunity and Vaccines
Co-Chairmen: [redacted] and B. Ivins

AVA and rPA vaccine potency and safety: comparisons in animals
(Ivins or [redacted]

Efficacy of AVA against vaccine-refractory strains
[redacted] or Ivins)

DNA vaccines
(eg. [redacted] CBER)
(and - [redacted] et al.)

PA-producing recombinant *B.anthracis* vaccines
[redacted]

LF-fusion proteins
[redacted]

Other experimental vaccines:
(Live attenuated vaccines) -
[redacted]

Correlates of immune protection
In vitro correlate/animal models
USAMRIID
DERA (CBD) [redacted]

Human immune responses to anthrax vaccine - [redacted]

The SCID mouse model-

#8: Prevention and Treatment [Or Human Vaccines and Treatments]
Chairmen: [redacted]

Anthrax vaccination: Safety and immunogenicity of alternate schedules and routes:
[redacted]

Surveillance of military personnel immunized with AVA:
[redacted]

Human Clinical studies with PAVAX
[redacted]

Human antitoxin antibodies
("scFV": [redacted] - Los Alamos)

Toxin competitive inhibitors

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("PA32": [redacted] - Los Alamos)

Update on Antibiotics: efficacy, resistance (lectures or posters)
[redacted] - USAMRIID)

CLOSING Remarks (Conference overview): [redacted]

file: 4th Anthrax Wkshp.Scient.Prog#2

4th International Conference on Anthrax

Annapolis, MD U.S.A.

10 - 13 June 2001

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Scientific Program [SUBJECT CATEGORIES and possible speakers and subjects]
REF.: Files (1)4th Anthrax Wkshp.reviewers (2) 4th Anthrax Wkshp.people

DAY 1:

Opening Remarks (1) [redacted] (USAMRIID);
and? - (2) [redacted] [or present talk during the main "Conference Dinner": "emeritus speaker"]

#1: Ecology and Incidence

Chairman: [redacted]

Anthrax and the internet: 15 min.
A balance between immediacy and accuracy of information
[redacted]

2001 Global Anthrax Report 15 min.
[redacted]

Updates - Foci and outbreaks of human anthrax (possible lectures or posters)
Central Asian republics
Russia
U.S.A.: [redacted] or CDC rep [redacted]
China

Updates: Foci and outbreaks in animals (possible lectures or posters)
Canada/USA: domesticated and wild animals
[redacted]?
Australia: Update [redacted]
Africa: Updates
[redacted] Namibia? [redacted] South Africa?

Late-breakers

#2: Risks and hazards

Chairman: [redacted]

Bioterrorism and the alleged use of anthrax:
Overview and management

OR: Anthrax as a biological weapon:
Medical and Public Health Management
[Working Group for Civilian Biofense:
Rep from JHU, DHHS/CDC, or USAMRIID (DOD - no?)
[redacted]

35 tapes, Phase II POIs
Ivins, Bruce . PSJ

Disposal of stockpiles in the FSU:
discovery of abandoned spore stockpiles in Uzbekistan/Kazahkstan
[A Russian - sensitivity issue?]

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#3: Detection, Identification, and Epidemiology of *B. anthracis*

Chairman: [redacted]

Genetic Diversity of *B. anthracis*
plasmid-specific diversity:
[redacted] (*pag* gene diversity)

chromosomal polymorphisms:
New markers and procedures
(MLVA/multiplex PCR)
vrr loci polymorphisms
[redacted]

Genetic markers for detection:
RAPD fingerprinting
[redacted]

Updates on other genetic markers (Lectures or Posters)
[redacted]
[redacted] (SASP gene probes and Bacillus evolution)?

Immunological detection:
Antibody-based systems of detection in natural samples
(NMRI- [redacted]

Monoclonal antibodies to spores and vegetative cells
[redacted]

Other methods for rapid detection (Lectures or Posters)
eg. [redacted] (NNRI); ribotyping
[redacted] (DARPA); molecular diagnostics and detectors

Chairman's Overview: Identification and Diversity of *B. anthracis*

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DAY 1 or 2:

#4: Molecular Biology and Genomics
Chairman: [redacted]

The genome of *B. anthracis* strain Ames: sequence and analysis
[redacted] TIGR)

Sequence analyses of pX01 and pX02: Updates
[redacted] etc.)

Characterization of plasmid replication sequences
[redacted]

DAY 2:

#5: Structure and Function:
Chairman: [redacted]
or [redacted]

Genetic Control of Sporulation and Germination OR
Genetc Control of Spore and Vegetative Cell Structure
(Review: [redacted]

Germination genes of *B. anthracis*:
[redacted] germination loci)
[redacted] group, germination loci)

Characteristics of the exosporium
(Update: [redacted])

Spore Structural Proteins - [OR in Topic #3]
[redacted]

S-Layer Homologous Domains of bacterial surface proteins
[redacted] group- [redacted]

#6: Pathogenesis and Genetic Regulation
Chairman: [redacted]

The *pag* operon: Regulation of *pagA* by *pagR*
[redacted]

Identification of receptor-binding regions in PA (two groups):
(1) [redacted]
(2) [redacted]

Oligomerization of PA and cellular uptake of toxin
[redacted]

Mechanism of anthrax toxin entry into cells
[redacted] (1) Characterization of the PA Channel
(2) A polarized epithelial cell model for toxin entry

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Role of the Proteosome in LF toxicity
[redacted] et al.)

Updates (lectures or posters)
LF Fusion -mediated delivery system
[redacted]

LF Mechanism of Action (MAPKKs, etc.)
[redacted] or group (eg., [redacted]

New candidate vaccines

The interaction of *B. anthracis* with macrophages in pathogenesis
[redacted]

Anti-spore host responses?
[redacted]

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DAY 3:

Alternate: Combine categories #7 and #8: Immunoprophylaxis and Treatment
Chairmen: [redacted]

#7: Vaccines and immune responses
Co-Chairmen: [redacted] and B. Ivins

AVA and rPA vaccine potency and safety: comparisons in animals
(Ivins or [redacted]

Efficacy of AVA against vaccine-refractory strains
[redacted] or Ivins)

DNA vaccines
(eg. [redacted] CBER)
(or - [redacted] et al.)

PA-producing recombinant *B.anthracis* vaccines
[redacted]

LF-fusion proteins
[redacted]

Other experimental vaccines:
(Live attenuated recombinant vaccine: [redacted]

Correlates of immune protection (possible subjects . .)
In vitro correlate/animal models
USAMRIID
DERA (CBD [redacted]

Human immune responses to anthrax vaccine - [redacted]

The SCID mouse model-

#8: Prevention and Treatment [Or Human Vaccines and Treatments]
Chairmen: [redacted]

Anthrax vaccination: Safety and immunogenicity of alternate schedules and routes:
(A. [redacted]

Surveillance of military personnel immunized with AVA:
[redacted]

Human Clinical studies with PAVAX
[redacted]

Human antitoxin antibodies
("scFV": [redacted] Los Alamos)

Toxin competitive inhibitors
("PA32": [redacted] - Los Alamos)

Update on Antibiotics: efficacy, resistance (lectures or posters)

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CLOSING Remarks (Conference overview): [redacted]

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file: 4th Anthrax Wkshp.Reviewers

4th International Conference on Anthrax: Abstract Review Committee

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TO: [redacted]

Bruce Ivins

[redacted]

SUBJECT: Program Development - Abstract Review and Selection

We need to develop a list of Subject Categories for placement of the abstracts that will be submitted to the Anthrax Conference. The abstracts will need to be reviewed and selected, and this will be done by a Program Committee.

We should first agree on a list of Subject Categories to which each abstract will be assigned, and forward this list to [redacted] our POC for the meeting at the ASM. The Subject Categories will then be posted on the Anthrax Conference web site that ASM will set up. The abstracts will be placed into the categories as they are submitted, so that the reviewers can download them.

[redacted] suggested that we ask two people that are prominent in each Subject Category to serve on the committee. We should agree upon a list of reviewers (with a few alternates, as backups for anyone that cannot participate). I'll send this list to [redacted] and [redacted] will send a letter to each of our selected individuals to ask them if they would be willing to serve as a reviewer. We might also consider calling some of the people directly to find out if they would be available.

Each Subject Category with its two reviewers will be a Subcommittee that will only be required to review and score for acceptability the abstracts that are submitted for that category. The abstracts will be retrieved by the reviewers from the Management section of the conference website. The abstracts could be scored by a scheme such as the following, based on scientific content and/or level of interest/topical nature): 0 = reject and 1 - 4 = consider for selection, with 1 = marginal and 4 = fully acceptable. We'll give [redacted] a deadline for completion of the reviews; and she'll post this on the web site.

After all the abstracts are are submitted and sent out for review, it would be good to have a general meeting of all the reviewers to: (1) decide which abstracts will be accepted; most will be accepted as poster presentations. (2) Which of the submitters of abstracts we'll invite to give an oral presentation (instead of poster) as part of the main program. We should also have a short list of alternates that could be contacted if the invited speaker is unable to give a presentation. The list of accepted abstracts will be given to [redacted] who will inform the submitters about the disposition of their abstract via the Conference web site (eg. accepted as a poster or rejected). The list of invited speakers will also be given to [redacted] who will send out an official invitation letter. If some of the invitees are unable to present orally, the alternate selection will be invited. We should also identify people to invite to chair sessions in the final program.

The following is a tentative list of Subject Categories and people who are prominent in the field and might be willing to serve as a reviewer. This is based on my guesstimates and limited range of contacts (see list below: Anthrax research - places and people).

PLEASE make any changes or additions to either the subject categories or reviewers. You can add your suggestions to this and email, or whatever works. I need your input!

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Subject Category*	Possible Reviewer
#1: Ecology and Incidence	[redacted]
#2: Risks and hazards	CDC: [redacted] USAMRIID: [redacted]
#3: Detection, Identification, and Epidemiology of *B. anthracis*	[redacted] DARPA: _____ NAMRI: _____
#4: Molecular Biology and Genomics	[redacted] TIGR: [redacted]
#5: Structure and Function:	[redacted] Pasteur: [redacted]
#6: Pathogenesis and Genetic Regulation	[redacted]
#7: Vaccine prophylaxis and immune responses	USAMRIID: Ivins, [redacted] [redacted] and colleagues: [redacted] Israelli group: [redacted]
#8: Prevention and Treatment	Los Alamos: [redacted]

*Reference: "4th International Conference on Anthrax" - [redacted] tentative Scientific Program

Anthrax research - places and people:

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Affiliation	point of contact: Subject
DARPA	[redacted] Detection, Identification,Epidemiology: molecular diagnostics
	[redacted] sp?):Detection,Identification,Epidemiology detectors
Pasteur Inst.	[redacted] and group [redacted] Pathogenesis and Genetic regulation regulation of plasmid-encoded virulence factors toxin structure and functional domains structure of bacterial surface proteins
	[redacted] Pathogenesis and Genetic regulation germination and the macrophage
	[redacted] Detection, Identification,Epidemiology multiplex PCR
NIH	[redacted] Vaccine group:
	[redacted] Pathogenesis and Genetic Regulation Identification of receptor-binding regions in PA Role of the Proteosome in LF toxicity LF Fusion -mediated delivery system LF Mechanism of Action (MAPKKs. etc
NAMRI:	
Naval Medical	?: Detection, Identification, Epidemiology
CDC	POC for Anthrax group = ?
	[redacted]
Porton/DERA, CBD	[redacted] vaccines vaccines Vaccines experimental vaccines (eg. DNA) correlates of immunity
	[redacted] Ecology and Incidence ?
	[redacted] Structure/Function Genetic control of sporulation/germination
Porton/CAMR	[redacted] Conference Summary - "emeritus" speaker?
Louisiana State	[redacted] Ecology and Incidence
Los Alamos	[redacted] Detection, Identification,Epidemiology molecular epidemiology
	[redacted] Prevention and Treatment

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human antibodies and toixn inhibitors

N. Arizona U. [redacted] Detection, Identification,Epidemiology
molecular epidemiology

TIGR [redacted] Molecular Biology and Genomics
sequence analysis of *B. anthracis* genome

NCI/FCRF POC?: Pathogenesis and Genetic regulation
LF Mechanism of Action (MAPKKs. etc

U. Houston: [redacted] Pathogenesis and Genetic regulation
regulation of the *pag* operon

U. Michigan [redacted]
[redacted] Pathogenesis and Genetic regulation
germination and the macrophage
pathogenesis and the macrophage

U. Michigan [redacted] Structure/Function
Genetic control of sporulation/germination

U. California [redacted] Structure/Function
Spore proteins

Tufts [redacted] Pathogenesis and Genetic regulation
Mechanism of anthrax toxin entry into cells

Canada Dept.
Renew.Res [redacted] Ecology and Incidence

Australia Dept.
Nat. Resources [redacted] Ecology and Incidence

USAMRIID Ivins, [redacted] Vaccine prophylaxis/immune responses
AVA vaccine potency, efficacy,
[redacted]
plasmid replication
LF-fusion vaccines

[redacted] Vaccine prophylaxis/immune responses
Recombinant *B. anthracis* vaccines
LF-fusion vaccines

[redacted] Vaccine prophylaxis/immune responses
Correlates of immunity

Russians [redacted]

[redacted]
etc.: [cannot be considered as reviewers/presenters]

Israelli Defence Dept. [redacted] Vaccines
PAVAX, experimental/live vaccines

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

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FEDERAL BUREAU OF INVESTIGATION

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Date of transcription 07/28/2005

Surveillance was conducted at BRUCE E. IVINS' home address, [redacted] on July 28, 2005 at approximately 8:15 a.m. The surveillance was performed in order to determine if there were any unusual vehicles or unusual activity at the home. No unusual activity was noted. Three vehicles previously known to be associated with IVINS were parked either in the driveway or along the curb in front of [redacted] These vehicles were:

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[redacted]

Investigation on 07/28/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID -1388 Date dictated

by [redacted]

This document contains neither recommendations nor conclusions of the FBI. It is the property of the FBI and is loaned to your agency; it and its contents are not to be distributed outside your agency.

279A-WF-222936-USAMRIID -1389
NPU:npu

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DATE 12-15-2008 BY 60324 UC BAW/DK/TH

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The following investigation was conducted by Special Agent [redacted] on August 3, 2005:

Writer performed a search for the name [redacted] in the United States Army Medical Research Institute of Infectious Diseases' (USAMRIID) key card access records for the time period from August 1998 through June 2002. The name [redacted] was not found in the key card access records for the abovementioned time period.

[Administrative: [redacted] collaborated with BRUCE EDWARDS IVINS on a experiment that took place at USAMRIID. [redacted] corresponded with IVINS via numerous emails during 1998.]

[redacted]

[redacted]

[redacted]

(Rev. 01-31-2003)

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FEDERAL BUREAU OF INVESTIGATION

Precedence: ROUTINE **Date:** 07/18/2005

To: Washington Field **Attn:** SSA [redacted]
SA [redacted]

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From: Washington Field
AMX-3
Contact: [redacted]

Approved By: [redacted]

Drafted By: [redacted]

Case ID #: 279A-WF-222936-USAMRIID (Pending) -1392

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Title: AMERITHRAX;
MAJOR CASE 184

Synopsis: To provide results of AMX-3 investigative lead to review the book Arrowsmith, by Sinclair Lewis.

Details: During the course of captioned investigation, Bruce Edwards Ivins of the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) was evaluated due to his anthrax expertise. Ivins read the book, Arrowsmith, by Sinclair Lewis in early high school, and has stated that this book ultimately led to his decision to become a scientist. The book, which details the fictional story of a scientist who tries to preserve his ideals in the face of societal corruption, was read to gain insight into Ivins' possible state of mind as a result of his reading the book. Pertinent excerpts from Arrowsmith follow:

> *Page 38*
> There is but one trouble with a philosophical bacteriologist. Why should we destroy these amiable pathogenic germs? Are we too sure, when we regard these oh, most unbeautiful young students attending YMCA's and singing dinkle-songs and wearing hats with initials burned into them - iss [sic] it worth while to protect them from the so elegantly functioning *Bacillus typhosus* with its lovely flagella? You know, I once asked Dean Silva would it not be better to let loose the pathogenic germs on the world, and so solve all economic questions.
>
> *Page 123*

[redacted]

[redacted]

He reflected (it was an international debate in which he was joined by a few and damned by many) that half a dozen generations nearly free from epidemics would produce a race so low in natural immunity that when a great plague, suddenly springing from almost-zero to a world-smothering cloud, appeared again, it might wipe out the world entire, so that the measures to save lives to which he lent his genius might in the end be the destruction of all human life. He meditated that if science and public hygiene did remove tuberculosis and the other major plagues, the world was grimly certain to become so overcrowded, to become such a universal slave-packed shambles, that all beauty and ease and wisdom would disappear in a famine-driven scamper for existence. Yet these speculations never checked his work. If the future became overcrowded, the future must by birth-control or otherwise look to itself. Perhaps it would, he reflected. But even this drop of wholesome optimism was lacking in his final doubts. For he doubted all progress of the intellect and the emotions, and he doubted, most of all, the superiority of divine mankind to the cheerful dogs, the infallibly graceful cats, the unmoral and unagitated and irreligious horses, the superbly adventuring seagulls.

Page 248
None of these novelties was so stirring as the Eugenic Family, who had volunteered to give, for a mere forty dollars a day, an example of the benefits of healthful practices. They were father, mother, and five children, all so beautiful and powerful that they had recently been presenting refined acrobatic exhibitions on the Chautauqua Circuit. None of them smoked, drank, spit upon pavements, used foul language, or ate meat.

Page 278
He is the only real revolutionary, the authentic scientist, because he alone knows how liddle [sic] he knows. He must be heartless. He lives in a cold, clear light. Yet dis [sic] is a funny t'ing [sic]: really, in private, he is not cold nor heartless - so much less cold than the Professional Optimists. The world has always been ruled by the Philanthropists: by the doctors that want to use therapeutic methods they do not understand, by the soldiers that want something to defend their country against, by the preachers that yearn to make everybody listen to them, by the kind manufacturers that love their workers, by the eloquent

statesmen and soft-hearted authors - and see once what a fine mess of hell they haf [sic] made of the world! Maybe now it is time for the scientist, who works and searches and never goes around howling how he loves everybody!

Page 347
There may have been in the shadowy heart of Max Gottlieb a diabolic insensibility to divine pity, to suffering humankind; there may have been mere resentment of the doctors who considered his science of value only as it was handy to advertising their business of healing; there may have been the obscure and passionate and unscrupulous demand of genius for privacy. Certainly he who had lived to study the methods of immunizing mankind against disease had little interest in actually using these methods. He was like a fabulous painter, so contemptuous of popular taste that after a lifetime of creation he should destroy everything he has done, lest it be marred and mocked by the dull eyes of the crowd.

Page 354
Be sure you do not let anything, not even your own good kind heart, spoil your experiment at St. Hubert. I do not make funnies about humanitarianism as I used to; sometimes now I t'ink [sic] the vulgar and contentious human race may yet have as much grace and good taste as the cats. But if this it to be, there must be knowledge. So many men, Martin, are kind and neighborly; so few have added to knowledge. You have the chance! You may be the man who ends all plague, and maybe old Max Gottlieb will have helped, too, *hein* [sic], maybe? You must not be just a good doctor at St. Hubert. You must pity, oh, so much the generation after generation yet to come that you can refuse to let yourself indulge in pity for the men you will see dying. Dying...It will be peace. Let nothing, neither beautiful pity nor fear of your own death, keep you from making this plague experiment complete.

Page 373-374
He had seen the suffering of the plague and he had (though he still resisted) been tempted to forget experimentation, to give up the possible saving of millions for the immediate saving of thousands.

> *Page 377*
> Beside him stood Max Gottlieb, and in Gottlieb's power he reverently sought to explain that mankind has ever given up eventual greatness because some crisis, some war or election or loyalty to a Messiah which at the moment seemed weighty, has choked the patient search for truth. He sought to explain that he could-perhaps-save half of a given district, but that to test for all time the value of phage, the other half must be left without it...though, he craftily told them, in any case the luckless half would receive as much care as at present.

As the above excerpts illustrate, Martin Arrowsmith, the protagonist, learns that, in order to ensure his experimental plague vaccine works, he must allow some people to die of plague instead of immunizing them. Arrowsmith's mentor, Max Gottlieb, teaches him that people have to die in the short run in order for the world as a whole to be saved in the long run. In addition, Gottlieb takes a cynical view of the world, and feels that it may not be worth saving in the first place, with its unlovable people and overcrowded conditions. This satirical novel presents Arrowsmith and Gottlieb's view of society and how they, as scientists, must be more loyal to the search for truth and scientific breakthroughs than to soft-heartedness and temporary solutions.

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Precedence: ROUTINE **Date:** 06/27/2005

To: Washington Field

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-15-2008 BY 60324 UC BAW/DK/TH

From: Washington Field
Amerithrax 3
Contact: SA [redacted]

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Approved By: [redacted]

Drafted By: [redacted]

Case ID #: 279A-WF-222936-USAMRIID (Pending)-1394

Title: AMERITHRAX;
MAJOR CASE 184

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Synopsis: To report on the analysis of USAMRIID keycard access database records corresponding to the July 2004 consensual search and inventory of *Bacillus anthracis* Ames strain material maintained at USAMRIID.

Details: On July 16, 2004 through July 23, 2004, the FBI's Amerithrax Taskforce and Hazardous Material Response Teams (HMRTs) conducted a consensual search and inventory of *Bacillus anthracis* (*B.a.*) Ames strain materials maintained in Buildings 1412 and 1425 of the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). The search and inventory were conducted to ensure full compliance with a previously issued subpoena requesting submission of all USAMRIID *B.a.* Ames strain isolates to the FBI Repository.

Prior to commencement of the search, an agreement was reached between the FBI and USAMRIID wherein personnel from both parties accessing the facility during the search period would have restricted and recorded access to areas within Buildings 1412 and 1425. Limited access was deemed necessary to conduct a safe, secure and thorough search, and was designed to minimize the impact to ongoing research at USAMRIID. An agreement was also reached that USAMRIID and FBI personnel would escort one another into the hot suites, or biocontainment areas, as a safety consideration and to maintain the integrity of the search.

The following 18 FBI HMRT personnel participated in the search and inventory of *B.a.* Ames within the USAMRIID hot suites in July 2004: [redacted]

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[redacted] These FBI personnel represented HMRTs from the Baltimore, Chicago, Detroit, Miami, Pittsburgh and Washington Field Divisions.

On January 19, 2005, [redacted] of USAMRIID's [redacted] work telephone [redacted] provided keycard access database records corresponding to the July 2004 search period. [redacted] furnished a CD-ROM, titled "Access Data for FBI: USAMRIID Access Records, July 16, 2004 through July 23, 2004." The compact disc contained the access records for all badge readers in USAMRIID Buildings 1412 and 1425, to include all hot suite keypad records. The database incorporated the access records of all FBI personnel and USAMRIID employees and contractors for the period of July 16, 2004 through July 23, 2004. The CD-ROM provided by [redacted] is maintained in the case file in a 1A envelope labeled "USAMRIID keycard access database records related to the July 2004 consensual search and inventory of *Bacillus anthracis* (*B.a.*) Ames strain materials maintained at USAMRIID: two 3½ inch diskettes and one CD-ROM disc."

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The USAMRIID access records were analyzed to assess the level of compliance with the pre-search agreement that all USAMRIID personnel accessing designated hot suites during the search period required accompaniment by FBI personnel. The electronic keycard access database was partitioned according to date. Excel spreadsheets, containing records of access for all keycard readers in Buildings 1412 and 1425, were generated for each day of the search. Keycard reader and keypad records associated with access the Building 1412 hot suite and Building 1425 suites [redacted] and [redacted] were evaluated, as these locations encompassed the areas where *B.a.* Ames was utilized and maintained at USAMRIID. Specifically, access records corresponding to the following locations were allotted particular scrutiny:

Building 1412

[redacted]
Male change room entry reader
Male change room exit reader
Hot suite entry keypad

[redacted]
Female change room entry reader
Female change room exit reader
Hot suite entry keypad

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Building 1425

[redacted] Male change room entry reader
Male change room exit reader
Hot suite entry keypad

[redacted] Female change room entry reader
Female change room exit reader
Hot suite entry keypad

[redacted] Male change room entry reader
Male change room exit reader
Hot suite entry keypad

[redacted] Female change room entry reader
Female change room exit reader
Hot suite entry keypad

[redacted] Male change room entry reader
Male change room exit reader
Hot suite entry keypad

[redacted] Female change room entry reader
Female change room exit reader
Hot suite entry keypad

Airlocks
1412 First floor airlock entry reader
1412 [redacted] airlock entry reader
1412 Basement airlock entry reader

1425 [redacted] airlock entry reader

1425 [redacted] airlock entry reader
1425 [redacted] airlock entry reader

Results of analysis

Evaluation of the access records revealed that, with a few exceptions, USAMRIID personnel accessing designated hot suite areas during the July 2004 search and inventory period were accompanied appropriately by FBI personnel. Following is a brief synopsis of the search time line, incorporating three instances in which direct escort of a USAMRIID employee by an FBI representative was not readily apparent. USAMRIID personnel are identified below with capital letters.

The consensual search and inventory of USAMRIID's entire collection of *B.a.* Ames was initiated on July 16 and completed on July 23. The first joint entry of USAMRIID and FBI personnel into a hot suite occurred in Building 1412 at

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12:35 pm on July 16. SA [redacted] accompanied [redacted] into the biocontainment area. The search of Building [redacted] ensued until July 21. With the exception of two instances, all entries of USAMRIID personnel into the Building [redacted] hot suite clearly corresponded to concurrent entries by FBI personnel.

On July 19 at 9:55 am, BRUCE IVINS entered the [redacted] male change room in Building 1412 and accessed the hot suite at 9:57 am. According to the access records, IVINS was not directly escorted by FBI personnel; however, numerous FBI and USAMRIID personnel were already present in the 1412 hot suite. SAs [redacted] entered the male change room at approximately [redacted] shortly after IVINS. IVINS' exit from the male change room at 11:16 am corresponded with the [redacted] am exits of [redacted] suggesting that IVINS' presence was known to the FBI personnel working in the Building [redacted] hot suite.

On July 20 [redacted] entered the [redacted] change room in Building 1412 and accessed the hot suite at [redacted] According to the access records, [redacted] was not directly escorted by a [redacted] HMRT member; however, numerous FBI and USAMRIID personnel were already present in the hot suite. [redacted] exited the [redacted] change room at [redacted] Notably, SAs [redacted] entered the [redacted] change room at [redacted] and exited at [redacted] [redacted] worked for [redacted] Writer recalled that SA [redacted] was responsible for leading an HMRT team in the inventorying of [redacted] spaces within the Building 1412 hot suite. Therefore, writer speculates that [redacted] was accompanied by SAs [redacted] during [redacted] tenure in the suite on [redacted]

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According to the FBI's Command Post Administrative Log, evidentiary items collected from Building 1412 were transported to the FBI refrigerator/freezer in the Building 1425 [redacted] hallway at 4:30 pm on July 21, 2004. Once the evidentiary items were removed from Building 1412 on July 21, the hot suite areas were released and authorized USAMRIID personnel were permitted to enter the biocontainment areas without accompanying FBI personnel.

On July 19, entry photography was conducted and sketches were generated for Building 1425 suites [redacted] and B3/B4. The search and inventory of *B.a.* Ames in Building 1425 ensued until July 23. Prior to initiation of the Building 1425 search, USAMRIID personnel were allowed to conduct essential work in the hot suites, provided that an FBI HMRT

member was present. With the exception of one instance, all entries of USAMRIID personnel into the Building 1425 hot suites from July 16 - 23, 2004, corresponded to concurrent entries by FBI personnel.

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On July 17 at approximately [redacted] entered the [redacted] change room. [redacted] gained access to the B4 suite via the [redacted] keypad at [redacted] A keypad code entry was not recorded for [redacted] [redacted] exited the [redacted] change room at [redacted] respectively. According to the access records, no FBI personnel were present in the B3/B4 suite during the early morning of [redacted] thus suggesting that [redacted] were not directly escorted by a male or female HMRT member. Considering that [redacted] was one of the initial days of the search and FBI personnel were new to USAMRIID protocols, writer speculates that an FBI escort was likely present, but did not badge into or out of the change room. USAMRIID identified [redacted] as requiring access to suite B3/B4 on [redacted] in a memorandum to the FBI, dated July 16, 2004, and titled "Access to 1412 (hot side), [redacted] and B3/B4." The memorandum further stated that, along with the individual needing access to the hot suite, a USAMRIID escort was required to accompany the FBI escort for safety monitoring. Writer speculates that [redacted] was the USAMRIID escort, while [redacted] conducted [redacted] work within suite B3/B4.

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By early afternoon of July 22, evidence collection and exit photography of suite [redacted] were completed. Inventory and evidence collection of suite B3/B4 was completed by 7:00 pm on July 23.

The Excel spreadsheets, created for each day of the search, are contained on a CD-ROM titled "USAMRIID keycard access data for July 16 - July 23, 2004." Hard copies of the spreadsheets and notes generated during the analysis are maintained, along with the CD-ROM, in a 1A envelope titled "Analysis of keycard access database records corresponding to the July 2004 consensual search and inventory of *B.a.* Ames strain material maintained at USAMRIID. Contains hard copies of access records, analysis notes, and a CD-ROM of electronic access records."

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