FBI ACCUSED IVINS OF HIDING MATERIAL WHILE FBI HID DATA FROM PUBLIC, IVINS' ATTORNEY

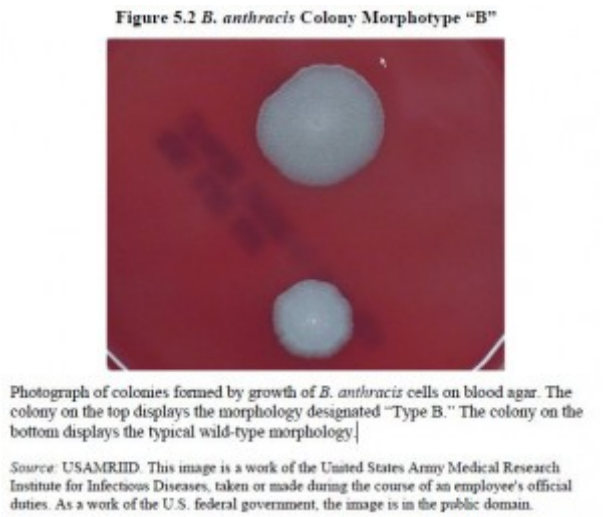

Figure 5.2 *B. anthracis* Colony Morphotype "B"

Photograph of colonies formed by growth of *B. anthracis* cells on blood agar. The colony on the top displays the morphology designated "Type B." The colony on the bottom displays the typical wild-type morphology.

Source: USAMRIID. This image is a work of the United States Army Medical Research Institute for Infectious Diseases, taken or made during the course of an employee's official duties. As a work of the U.S. federal government, the image is in the public domain.

A huge portion of the FBI's circumstantial case against Bruce Ivins in the Amerithrax investigation of the 2001 anthrax attacks relies on the scientific analysis carried out to provide a genetic fingerprint of the anthrax spores in Ivins' RMR-1029 flask as the source from which the attack material was cultured. One of the central supporting pieces of evidence the FBI touts in this regard is the claim that Ivins submitted a sample to the FBI in April of 2002, labeled as arising from the RMR-1029 flask, but missing the key genetic variants which the FBI used to characterize the material in RMR-1029. Through diligent analysis of thousands of pages of FBI files, a team consisting of McClatchy, ProPublica and Frontline has found that the FBI has not been entirely forthcoming about samples submitted to them by Ivins:

> Prosecutors have said Ivins tried to hide his guilt by submitting a set of false samples of his Dugway spores in April 2002. Tests on those samples didn't display the telltale genetic variants later found in the attack

> powder and in sampling from Ivins' Dugway flask.
>
> Yet records discovered by "Frontline," McClatchy and ProPublica reveal publicly for the first time that Ivins made available at least three other samples that the investigation ultimately found to contain the crucial variants, including one after he allegedly tried to deceive investigators with the April submission.
>
> Paul Kemp, who was Ivins' lawyer, said the government never told him about two of the samples, a discovery he called "incredible." The fact that the FBI had multiple samples of Ivins' spores that genetically matched anthrax in the letters, Kemp said, debunks the charge that the biologist was trying to cover his tracks.

As a ProPublica article piles onto the material above from McClatchy, the lead prosecutor in the case continues to claim that the one sample lacking variants is a strong indicator of Ivins' guilt and shows that he tried to hide the RMR-1029 flask from further scrutiny:

> Rachel Lieber, the lead prosecutor in a case that will never go to trial, thinks that Ivins manipulated his sample to cover his tracks.
> "If you send something that is supposed to be from the murder weapon, but you send something that doesn't match, that's the ultimate act of deception. That's why it's so important," Lieber said.

But did Ivins really manipulate the sample? That is not entirely clear, especially when the microbiology and genetics relevant to the situation are considered along with the new knowledge that three other samples submitted by

Ivins did have all of the genetic variants present.

The photo above comes from the National Academy of Science report on their investigation into the scientific approach taken by the FBI in the Amerithrax investigation. The photo shows the subtle difference in the growth habit on agar for a colony arising from a single normal cell (bottom) and a colony arising from a single variant cell (top). For their analysis, the FBI developed DNA tests that could distinguish four specific mutations that could produce four of the colony variants observed. It should be noted that the FBI found that in some cases, more than one different DNA change within the same gene could produce the same apparent colony shape variant, but they chose a single DNA change to track for each colony variant.

What needs to be kept in mind is that these colony variants are present at a low concentration in RMR-1029. As the National Academy report described in its finding 5.5, the analysis did not address the relative abundance of the various DNA types in either the RMR-1029 reference material or any of the investigative samples:

> Finding 5.5: Specific molecular assays were developed for some of the B. anthracis Ames genotypes (those designated A1, A3, D, and E) found in the letters. These assays provided a useful approach for assessing possible relationships among the populations of B. anthracis spores in the letters and in samples that were subsequently collected for the FBI Repository (see also Chapter 6). However, more could have been done to determine the performance characteristics of these assays. In addition, the assays did not measure the relative abundance of the variant morphotype mutations, which might have been valuable and could be important in future investigations.

Keep in mind that RMR-1029 contained material produced in multiple fermenter runs at Dugway and a number of flask cultures at USAMRIID. Each individual culture that went into the RMR-1029 had the potential to produce its own spectrum of randomly arising DNA mutations which could have manifested as one of the colony variants chosen for analysis. Note also that the attack material was produced in one or more cultures presumably initiated with material arising from RMR-1029. The way in which the "starter" material was removed from RMR-1029 and how it was used to start the attack culture(s) would determine which variants were carried along, and in what ratios to one another and to the "normal" type. Furthermore, the conditions under which the attack cultures were produced would affect the final spectrum of variants present in the attack spore preparation.

Generally, microbiologists contend with the issue of randomly arising mutations by starting new cultures from a colony derived from a single cell from an older culture. This is achieved most often through use of a "streak plate" such as this one from Wikipedia:

A bacterial streak plate used to isolate colonies from single cells.

To produce such a plate, the microbiologist

starts with a liquid suspension of the old culture and dips into it a small sterilized wire loop which brings along with it a very small sample of the culture. The loop is then rubbed lightly over a small portion of the surface of a nutrient agar plate. The loop is then lifted off the surface of the agar, the plate rotated a few degrees, and the loop is rubbed lightly over the agar surface again, overlapping with the original area that received the liquid from the starter culture. This process is repeated several more times. After the plate is incubated for an appropriate amount of time, the pattern seen in the photo emerges. Because the concentration of bacteria in the starter culture is high, the region of the plate receiving the liquid directly from the starter culture is completely covered with a "lawn" of bacteria. As the starter bacteria are diluted with the successive rotations of the plate, individual colonies become apparent. The larger colonies separated by relatively large distances from one another can safely be assumed to have started from individual cells being deposited on the agar by the loop.

With that as background, now we can turn to the issue of the samples from RMR-1029 that Ivins provided to the FBI. The actual text of the sample preparation instructions in the subpoena under which Ivins and other researchers were ordered to submit samples is included on pages 76 and 77 of the Amerithrax report:

> 1. Collect each B. anthracis Ames strain stock as per your institutional inventory and personal knowledge.
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> 2. Prepare a minimum of two TSA [tryptic soy agar] slant tubes per stock by prelabeling with permanent waterproof labels. Include the following information on the label: "B. anthracis Ames strain," with other designators used by your laboratory, date and your lab name. Additional information for each stock shall be provided separately.

> 3. A representative sample of each stock shall be used for inoculation of the TSA slants. If the stock is an agar culture, do not use a single colony, but rather use an inoculum taken across multiple colonies. Thawed frozen stocks or other liquid suspensions shall be well mixed prior to transfer of inoculum to the TSA.
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> 4. Inoculate each TSA slant in a zig zag manner over the surface of the agar.
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> 5. Incubate the slants at 35ºC – 37ºC for 12-18 hr to confirm culture growth.
>
> 6. Individually wrap the slants in packaging materials approved for shipment of infectious select agents in accordance with regulations for the shipment of such materials.

The subpoena went to USAMRIID on February 15, 2002 and on February 27 Ivins prepared and submitted a set of samples. However, on March 28, those samples were rejected by the FBI. From page 78 of the Amerithrax report:

> On or before March 28, 2002 – the date the FBIR was officially up and running and had received its first sample, FBIR001 -Dr. Ezzell's lab technician advised Dr. Ivins and his lab technician that their submissions were not prepared according to the protocol. Specifically, Dr. Ivins and his lab technician used homemade slants as opposed to the commercially available Remel slants specified by the protocol, so the four slants prepared on February 27, 2002 were rejected by the FBIR, and Dr. Ivins was told to resubmit his culture samples on the appropriate slants.

Note that the portion of the protocol that the FBI put into the Amerithrax report did not mention that the TSA slant tubes had to be

commercially prepared rather than homemade. Tryptic soy agar is one of the most widely used culture media in microbiology and it is not at all uncommon for researchers to prepare their own slants, as many laboratories go through very large volumes of both petri dishes and slants with TSA.

Ivins resubmitted samples on April 10. From the ProPublica article:

> In April 2002, Ivins prepared a third sample from RMR-1029. This time, his lawyer said, he plucked a sample using a technique called a "single colony pick," a method biologists use to maintain purity when growing bacteria. Ultimately, this sample tested negative for the morphs. Prosecutors said they're not even sure that the sample Ivins submitted came from the flask. If it did, they said, he obstructed justice, since their subpoena instructed scientists to capture diverse samples of spores that would be sure to reproduce any morphs. Ivins told investigators he'd followed standard procedures for microbiologists when he sampled just one colony.

The Amerithrax report is vague about just what instructions, if any, were provided to Ivins when he was preparing his original sample:

> On February 27, 2002, one of the FBI Special Agents heading up the scientific side of the investigation received a telephone call from Dr. Ivins regarding the submission. This agent no longer has an independent recollection of the telephone call from Ivins, but his contemporaneous notes from the call reflected that Dr. Ivins identified himself as a research microbiologist and provided his telephone number and facsimile number. Dr. Ivins also identified which cultures of B.

> anthracis he had in his possession, though RMR-1029 was not listed. One of the cultures noted, however, was “1987 spores fm Dugway,” which is likely a reference to RMR-1029 with an incorrect date of 1987 instead of 1997. The agent noted: “will set up slants per subpoena today,” referencing Dr. Ivins. Given the notation of Dr. Ivins’s fax number and this statement, this agent believes that he faxed the protocol to Dr. Ivins that day for use in preparing his submissions.

Again, it seems important to me that the version of the protocol the FBI chose to insert into this section of the Amerithrax report does not have the instruction to use a commercial TSA slant. Is there another version of the protocol? Was that other version in the subpoena itself? [I will attempt to track down the actual subpoena, but the FBI document dump is not indexed.] Depending on how carefully Ivins reviewed the protocol instructions in April for his resubmission, and possibly which version of the protocol he may have reviewed, it is not all that surprising Ivins would rely on a single colony isolate for the RMR-1029 sample he submitted. Admittedly, the instructions in the Amerithrax report specifically state “liquid suspensions shall be well mixed prior to transfer of inoculum” and RMR-1029 was a highly concentrated liquid suspension. However, the same section also states “If the stock is an agar culture, do not use a single colony, but rather use an inoculum taken across multiple colonies.” This part is really sloppy, as “multiple colonies” normally would be interpreted to be as few as three or four and most likely not more than ten. Sampling in this way would be very likely to miss most if not all of the morphological variants present at low concentration, so sampling “multiple colonies” in this way would almost certainly give the same result as picking a single colony, is Ivins is believed to have done.

The ProPublica article points out that just before he submitted the homemade slant, Ivins had been discussing with the FBI the possibility of using DNA analysis to type the morphological variants and to use that information as a tool in identifying the source of the material used in the attacks. Note that this first sample he submitted after the discussion had all the variants present, but was rejected by the FBI. Although we will never know why Ivins used a single colony for the April submission, it could be as simple as him being busy and not looking back carefully at the instructions. It also is very likely that Ivins (and the other researchers submitting samples) was not told the exact nature of the analyses to be carried out. The DNA typing that eventually was carried out along the lines that Ivins had suggested above had not yet been developed in 2002 when he submitted this sample. If he suspected that DNA analysis was to be carried out, using a single colony would have been the logical choice, since a mixed population could produce ambiguous results in DNA sequencing. However, the fact remains that three out of four samples the FBI got from Ivins had the morphological variants present, so their continued insistence that the one sample lacking them is evidence of his guilt is hard to fathom.