

FEDERAL BUREAU OF INVESTIGATION

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**PRESS CONFERENCE FOR SCIENTIFIC MEDIA
WITH DR. VAHID MAJIDI, ASSISTANT DIRECTOR OF THE
FBI WEAPONS OF MASS DESTRUCTION DIRECTORATE
AND DR. D. CHRISTIAN HASSELL, FBI LABORATORY DIRECTOR
REGARDING THE SCIENCE OF THE ANTHRAX INVESTIGATION**

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Office of Public Affairs
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MR. KORTAN: My name is Mike Kortan from the FBI Office of Public Affairs, and I'm going to turn it over to Dr. Majidi here in a moment. Just to remind everybody, it's an on-the-record availability here as far as the two principles here, which we've described. And everyone else will be a background official that will support them.

We just remind everybody to use the microphones if you can when it gets to the question and answer point. The format's going to be Dr. Majidi is going to start off with an opening statement, an overview here. Dr. Hassell will introduce the panel members. And then we can begin a dialogue after that.

So Dr. Majidi.

DR. MAJIDI: Thank you. Good morning, ladies and gentlemen. I'm Vahid Majidi, the assistant director responsible for the FBI's Weapons of Mass Destruction Directorate. I would like to start today's session with a brief opening statement to define the scope of our roundtable discussion.

After nearly seven years of investigation, we have developed a body of powerful evidence that allows us to conclude that we have identified the origin and the perpetrator of the 2001 *Bacillus anthracis* mailing. The attribution process and identification of a specific perpetrator relies on the confluence of the intelligence, investigation and forensic information.

It is the information from forensic data that determined the source of the 2001 *Bacillus anthracis* mailing was derived from the spore preparation known as RMR 1029 that was maintained at U.S. Army Medical Research Institute for Infectious Disease, Fort Detrick, Maryland. From now on I'll refer to that as USAMRIID.

While there were countless investigative hours spent narrowing the field of suspects, we are here today to focus on the scientific aspects of this case. First of all, let me dispel some frequently repeated erroneous information.

For example, there were no intentional additives combined with the *Bacillus anthracis* spores to make them any more dispersible. Also, the purity of samples obtained from four letters were sufficiently different which allowed us to conclude that at least two different *Bacillus anthracis* batches were prepared from the original RMR 1029. This indicates that aliquots of RMR 1029 were removed and cultured in at least two separate batches to produce the materials used in the mailings.

The FBI began this complex investigation by coordinating analysis of spore powders contained in the 2001 *Bacillus anthracis* mailings. We enlisted the help of many biodefense experts to assist our examinations, including those who had previously developed tests to differentiate strains of *Bacillus anthracis* and identify the spores in the letters as the AMES strain.

Other analytical strategies were employed to target the chemical and elemental profile of the spore powders. Specific techniques include: scanning and transmission electron microscopy, energy dispersive x-ray analysis, carbon dating by accelerator mass spectrometry, and inductively coupled plasma optical emission and mass spectrometry.

Additional scientists from the Department of Defense and the Centers for Disease Control examined the spore material, and it was determined that there were many phenotypic variants within the samples. With generous support by both the National Institutes of Health and the National Science Foundation and other government agencies, FBI scientists worked with the Institute for Genomic Research to determine if genetic mutations were responsible for the altered appearance of the variants found in the *Bacillus anthracis* letters.

Several genetic mutants were discovered in these studies. FBI microbiologists contracted the assistance of several laboratories to develop highly specific assays to detect four specific genetic mutations found in the *Bacillus anthracis* letters. The mutation detection assays were validated and used by the FBI Laboratory to examine the repository of *Bacillus anthracis* AMES that was collected through the course of this investigation.

This unprecedented scientific approach allowed the FBI to identify the potential sources of the *Bacillus anthracis* used to produce the 2001 spore powders, and through a comprehensive analytical approach, the investigators were provided with validated scientific data which linked the material used in the 2001 attack to materials from USAMRIID identified as RMR 1029.

It is important to emphasize that the science used in this case is highly validated and well accepted through the scientific community. The novelty is the application of these techniques for forensic microbiology. Today I'm very confident that the significant lessons learned from the 2001 Bacillus anthracis case have been rigorously evaluated by the FBI, and appropriate actions have been taken to safeguard the American public.

The FBI Laboratory has revolutionized the approach to nontraditional forensic samples, and has developed robust capabilities to collect and examine evidence containing biological, chemical, radiological, or nuclear materials. We have developed a strong partnership with the U.S. government laboratory complex, public health system, private industry, and academia to significantly enhance our capabilities dealing with future investigations.

The creation of the Weapon of Mass Destruction Directorate is another example of FBI's progressive approach focusing on prevention as well as investigations on all issues involving chemical, biological, radiological, and nuclear materials. Please note that there were many dedicated individuals, including prosecutors, scientists, investigators, analysts, support personnel, that all worked on this case. For the purpose of this meeting, however, the science and technology community within the Bureau has the lead, and if individuals from the sidelines are asked to provide additional background to any of your questions, please make sure to attribute all answers to the FBI Laboratory Director, Dr. Hassell, sitting to my left.

Finally, I'm asking you to understand that this is the first step toward broader dissemination of scientific information surrounding this case. Additional information will be available through peer review publication, and I ask you to please respect the integrity of this process. In fact, several research projects related to the FBI's investigation have already resulted in peer review publications, and we will provide you with that list. Additional publications will be available for peer review as more information from the investigation is released.

Before we open the floor for a question-and-answer session, we would like to introduce you to our distinguished panel. Today we have with us a small group of individuals representing the large cadre of non-Bureau scientists that helped us chart and navigate our scientific path through this unprecedented case. In the near future, after we work through each nondisclosure agreement and privacy issues, we will release the names of those key individuals who tirelessly worked with us on the 2001 Bacillus anthracis mailing.

To my left is the current FBI Laboratory Director, Dr. Chris Hassell. Dr. Chris Hassell will introduce the panel members.

DR. HASSELL: Starting from my far left, we have Dr. Joseph Michael. He's a distinguished member of the technical staff at Sandia National Laboratories in Albuquerque, New Mexico. He currently works in the Materials Characterization Department of the Materials Science Center, where he develops and applies electron and

ion microscopy to the characterization of materials. Dr. Michael is the co-author of a leading textbook on scanning electron microscopy. He assisted with elemental analysis, electron microscopy of the samples, and with the development of the strategies for analysis of the chemical and physical characteristics of the spore powders.

Next on the panel is Dr. Jacques Ravel. He's an associate professor of microbiology and a member of the Institute for Genome Studies at the University of Maryland School of Medicine. He also was formerly with the Institute for Genomic Research. His research focuses on the application of microbial genomics to several key areas, including microbial genome sequence comparative analyses, with a special emphasis on human microbial pathogens, including *Bacillus anthracis*. His work included genetic sequence analysis and characterization of genetic mutants in support of the FBI investigation.

Next, to my immediate left, is Professor Claire Fraser-Liggett. She's a professor of medicine and director of the newly created Institute for Genome Studies at the School of Medicine, University of Maryland in Baltimore, Maryland. She was previously the president and director of the Institute for Genome Studies, where she holds -- excuse me. I'm sorry -- where she led teams that sequenced the genomes of several microbial organisms, including important human and animal pathogens. This institute performed a genetic sequence analysis in support of the anthrax investigation.

To the right of Dr. Majidi is Dr. Rita COLWELL. She is currently a distinguished professor both at the University of Maryland, College Park, as well as at the Johns Hopkins University Bloomberg School of Public Health. She's also a senior advisor to Canon U.S. Life Sciences, Incorporated. From 1998 to 2004, she served as director of the National Science Foundation, which provided funding for much of the genetic sequencing efforts in support of the FBI investigation. She has served as president of the American Association for the Advancement of Science, the American Society for Microbiology, and she's a member of the National Academy of Sciences. In July of 2007, she received the National Medal of Science.

Next on the panel is Dr. James Burans. He is currently the associate laboratory director of the National Bioforensic Analysis Center. He's been in the forefront of the development of diagnostic assay techniques to identify and characterize biological threat agents. He led several scientific working groups that were assembled from the National Academy of Sciences, National Laboratories, and other federal R&D facilities.

And last, to my far right, is Dr. Paul Keim. Dr. Keim is Regents Professor of Biology, and he holds the Cowden Endowed Chair in Microbiology at Northern Arizona University. He's also director of the Pathogen Genomics Division at the Translational Genomics Research Institute. His research focuses on molecular genetics for a wide variety of organisms, including bacteria, fungi, and plants and animals. His work in support of the FBI included identification of the spore powders as the AMES strain of the *Bacillus anthracis*.

And like Dr. Majidi mentioned, behind me are many of the staff members from the FBI

Laboratory who played a tremendous role in supporting this work, too. So they should be recognized as well.

DR. MAJIDI: The way we will work this morning's session is that I will ask each panel member to introduce themselves briefly, and give you an overview of exactly what area they worked on. After we are done, then the floor is open to questions from you. And I will be the moderator of this session. We'll try to focus all questions with regard to the science and technology aspects of this case only.

DR. MICHAEL: My name is Joe Michael. My area of expertise is electron microscopy. We apply electron microscopy to a range of cells to categorize materials. And our involvement was with respect to the physical chemical analysis of the attack materials.

DR. RAVEL: My name is Jacques Ravel. I'm an associate professor of microbiology at the Institute for Genome Sciences at the University of Maryland School of Medicine. Back in 2002, I was at the Institute for Genomic Research, also known as TIGR, where we as a team took on the responsibility to sequence some of the isolates of *Bacillus anthracis* that were provided to us by the FBI. We then sequenced them and kerotyped them genetically to find any kind of a genetic difference between the strain that we sequenced at the same time, which is also known as the AMES ancestor strain. And we used those genetic differences to design some of the assays that were used in the investigation.

DR. FRASER-LIGGETT: I'm Claire Fraser-Liggett, currently professor of medicine and director of the Institute for Genome Sciences at the University of Maryland School of Medicine. I was previously the director of the Institute for Genomic Research. Since 1995, I've been involved in comparative microbial genomic studies, and with my colleagues at TIGR, including Dr. Jacques Ravel, have been involved in the development of a number of experimental and computational approaches for identifying and characterizing differences in sequence composition and functional characterization of those sequence variants in a number of human and animal pathogens and environmental organisms.

In October 2001, at the time of the letter mailings, we were finishing up the first study to sequence what at the time was the first reference sequence of *Bacillus anthracis* AMES. It was an isolate that had been provided to us by Porton Down in the U.K. And as Jacques has described to you, following the letter attacks, our participation in the Amerithrax investigation expanded to begin to look at a number of additional samples and to begin to make genotype/phenotype characterizations.

DR. HASSELL: Thank you. Rita?

DR. COLWELL: I'm Rita COLWELL, a microbial systematist/microbial ecologist with a keen interest in molecular systematics. At the time of the anthrax incidents, I was the director of the National Science Foundation, and we had already a program in sequencing microbial genomes. It was clear to me that we must sequence the anthrax strains as soon

as possible, and with the small grants for innovative research were able to respond to a proposal submitted by TIGR to fund the sequencing. The continuation of the activity was through a consortium of agencies -- NIH, DOE, Justice, FBI, et cetera -- focused on ensuring that sequencing of pathogens would continue, notably, the anthrax strains.

DR. BURANS: My name is Jim Burans. I'm a retired naval officer. I come from the Navy's biodefense community. During the early '90s, I helped to support some of the early work with the FBI on biocrime investigations as well as supporting forensic analysis for the U.N. Special Commission to Iraq. In the November 2001 time frame, I was asked to serve as a scientific consultant in the early stages of the Amerithrax case. And in the 2003 time frame, I helped to establish the National Bioforensic Analysis Center for the Department of Homeland Security to deal with the scientific investigative challenges of biocrime and bioterror investigations.

DR. KEIM: I'm Paul Keim. I'm a professor of microbiology at Northern Arizona University. I'm also director of the Pathogen Genomics Division at the Translational Research Institute in Arizona. And I'm an affiliate at Los Alamos National Laboratory. My role in this is that prior to 2001, we'd begun studying *Bacillus anthracis* and its worldwide population diversity. We developed techniques to DNA fingerprint at what now seem a rather crude level but was very highly sophisticated at the time, such that in October 2001, the Bureau came to us to identify the strain that was in the anthrax letters.

And we did identify the AMES strain. We spent a lot of time after that trying to understand exactly what the AMES strain was and what it wasn't. Using DNA sequences from TIGR, we were able to develop very precise identification of the AMES strain itself, going back into nature to characterize natural populations as well as many different laboratory isolates. We were responsible for doing a lot of the biosafety handling in the early days of the investigation. We acted as a repository for the strains that were subpoenaed and collected across the country, characterizing those as AMES or not AMES. And recently we've been involved with preparing DNAs in our biosafety laboratory that were then sequenced at TIGR.

DR. MAJIDI: Thank you, Paul. I just want to point out that we specifically sought you out because you are involved with well-known, well-respected scientific journals. And we wanted to give you ample time to ask as many detailed questions with regard to the science of this case. We definitely didn't want your voice to get lost in the signal-to-noise ratio of all the other journals. So this is your time, and I'll open the floor to questions.

QUESTION: Yudhijit Bhattacharjee from Science Magazine. Before we actually get into the details, I just wanted to ask you, Dr. Majidi, to list what you characterize as the scientific evidence in this case.

DR. MAJIDI: We looked at a number of different characteristics of the samples. And ultimately, what really drove us to RMR 1029 was the ability to sequence and identify genetics variation that resulted in different phenotypes which unequivocally took us to RMR 1029. We have obviously done a number of other analyses, elemental

characterization, that drove us to conclude that there were no additives. We did various types of chemical analyses. And again, the techniques used were standard analytical laboratory techniques as well as various forms of microscopy. By and large, what drives us to RMR 1029 is the genetics information.

QUESTION: Would you take us on a step-by-step tour, starting from the sample in the letters to the flask at USAMRIID?

DR. HASSELL: This may be a group answer for this one. But let me step back also in the earlier question. Part of what we did was develop the process first before we really started into working on the samples themselves. So we got the panel members here and many others involved in setting out, if you have the evidence, what do you do with it? What's the most efficient way of analyzing? What's the most comprehensive way of making those determinations? So many, many discussions were held back in 2001 with several different working groups to do this. So we laid out the genetic way we would analyze it and the chemical, the physical methods there. So we put the process in place before we started doing anything, and we validated that process with the consensus of those scientists.

So because everyone here participated on this, I'm going to look for corrections as I go along here. But the samples were submitted for strain identification. That was the earliest step. And Dr. Keim was involved with that. Maybe you could give more detail and you could answer that?

BACKGROUND OFFICIAL: Yes. So the methods in 2001 were based upon hypervariable sequences that mutate very fast. *Bacillus anthracis* is a recently emerged clone, and so its genome is highly homogeneous compared to certain other pathogens. In the pre-genomics era, that was the way that we could do things. We could focus in upon these highly variable regions to get information about what a strain was and what it wasn't.

So we began characterizing it in that fashion. When the anthrax letters went out and we began receiving -- essentially every time a letter went out and isolates and a victim occurred, we received those isolates. And they would come to Flagstaff, where we would type them, subtype them with this approach. As the genomics came online and we began identifying single nucleotide polymorphism SNPs, those then became really the assay for identifying what the AMES strain was and what it wasn't. That method, we published that in EID and in other places, so those papers will be made available at some point. But those methods ended up being incredibly accurate and precise and robust, very sensitive. And again, they were able to identify what the AMES strain was and what it wasn't. Maybe something that is lost in all this is there were many false alarms over the last seven years of anthrax outbreaks, and that these assays were able to limit the investigation and focus in upon what really was the AMES strain. And it allowed the federal government to not worry about things that were not the AMES strain.

DR. HASSELL: Thank you. And following that, there were some other studies to look

at sort of the phenotypic variations within the samples. So we saw some variations in the different colonies that were unique to a certain subset of the samples that we had -- I'm sorry, to all of the evidentiary samples. They were common to all of those. And they were also found to be within a subset of the repository samples that we received from all the other laboratories.

We saw those phenotypic differences, like the difference in appearance, in how the colonies were looking and growing. Then we characterized those more fully and found the genetic mutations that were contained there. And then maybe I can turn it over to TIGR. Can you describe more what happened after that with characterizing those mutations?

BACKGROUND OFFICIAL: Yes. I think, again, just to step back, to put some of this later work in context, as I made reference to in my opening statement, with initial funding from the Office of Naval Research in 2001, we were completing the work on the first *Bacillus anthracis* genome sequence. And this project was to take that genome sequence, to complete closure where every base pair was determined without ambiguity. And we were very close to being done with that in October of 2001.

At the time of the anthrax letter attacks, as Dr. Colwell mentioned, we applied to NSF for SGER funding to do an initial comparison, which we completed very quickly and published in *Science* in 2002. I think that this was very important. We already knew from the work that had been done in Dr. Keim's lab that this was also a sample of *Bacillus anthracis* AMES. I think this was a very important first piece of information that set some of the strategy for this investigation going forward because this demonstrated to us that it was possible to identify high quality, reproducible polymorphisms when comparing two different samples of *Bacillus anthracis* AMES. And so that was very important information going forward. Had we done this comparison of two different samples of *Bacillus anthracis* AMES and found no differences whatsoever, I think we might have reconsidered the feasibility of going forward with the approach that we had taken. But we were very much encouraged with the genomics-based approach and its potential to reveal important information.

And as Paul has just described, then, we began to get samples that had come from the spore powders that had been collected as part of the ongoing investigation, and we took each of these through in terms of our routine sequence analysis process. As part of this, Jacques and some of his colleagues at TIGR developed a specific bioinformatics pipeline to rapidly identify potential polymorphisms. Again, one of the important things that we learned from our initial comparative study was that it was absolutely essential to be working with very high quality draft sequence, at a minimum, in order to reliably identify potential polymorphisms. We were able to convince ourselves, as part of the initial study, that low quality draft sequence could end up being a distraction because that were a sufficient number of sequencing errors, if you were working with low quality sequence, that could become a distraction. And we ended up -- you could end up chasing sequencing errors that turned out to be errors and not true polymorphisms.

So we were, as part of our initial studies, devising experimental and computational methods that allowed us to be much more efficient in our subsequent efforts. But as was made in Dr. Majidi's opening remarks or Dr. Hassell's remarks, the science that we were doing was -- we were not using new science. We were using science that we had had in place and had validated for many previous genome studies. We were now just applying this in a different way, with an eye towards identifying polymorphisms, sequence variants among the different samples of AMES that we were receiving.

DR. HASSELL: So then, just to bring us full circle, so then when the assays were developed, at the same time we were building a repository of samples from the whole population, or we sometimes use the term "universe," of people who had the AMES strain. We had that. We applied these assays to that. And there were really four assays that were developed that were highly specific. And I think it was just over a thousand samples. Within those samples, eight matched all four of those genetic markers. They were the markers for the mutation. Those eight are each traceable back from the investigation side back to RMR 1029. So finally, then, those four markers were also in all the samples from the evidence, from the letters themselves.

BACKGROUND OFFICIAL: I would like to add that there was an active consortium of agencies -- NSF, NIH, DOE, the intelligence community, Department of Justice, FBI, USDA, and DOD -- which acted as a source of advice. And this was a highly collaborative effort.

QUESTION: So these eight isolates that had all of the four mutations, you say that they were traceable to USAMRIID. Could you explain to me what that means?

DR. HASSELL: They were traceable through the investigative process. The science showed that they contained the markers.

QUESTION: I think there's --

DR. HASSELL: Well, I'm just saying that the way -- when we found out where those samples came from, they're from the individuals in the community who had those. That's where the investigation kicked in to say how they were related, how they were originally traced back in the investigation.

QUESTION: So it was more traditional police work that basically led you to believe that --

DR. MAJIDI: So let me go back to my original assertion, that any attribution is a confluence of three events: forensic information, investigative information, and intelligence information. So you can't really parse any one of these things and say which one really gave you that one last item. By tracing back to the RMR 1029, what we say is that through various mechanisms, we were able to validate that the samples had their origin from RMR 1029. We did that in a number of different methods. I don't want to go through all the specifics of the investigations because that's not the focus, but the simple

check of a lab notebook would be one way to do it. The shipment records would be another way to do it. There's a number of methodologies which is not that focused in this discussion today.

BACKGROUND OFFICIAL: I heard a misconception in your question and that was, you used the word "isolate" whereas you just used the word "sample," okay, and so when you think of these, you need to think of them as a collection of spores and that they contain these spore mutations that are being assayed for. An isolate would be derived from a single cell, for example, and it might not contain all four. It could be the collection or the subpopulation that contains it.

QUESTION: Yeah. No, I hope I can be forgive that because I'm actually quoting from the affidavit which uses the term "1,000 isolates." So I suppose instead of isolates that should really be 1,000 samples stored at more locations.

DR. MAJIDI: That's why we are here today.

QUESTION: Sure.

DR. MAJIDI: Because there are some issues.

QUESTION: No, sure. I mean, I --

DR. MAJIDI: Your point is well taken and we'll try to answer as many of those as we can, yes.

QUESTION: And so just to finish up that strain of --

DR. MAJIDI: Sure.

QUESTION: -- of questioning, if you will, --

DR. MAJIDI: Sure.

QUESTION: -- so these eight -- the four mutations were found in eight of the 1,000 samples --

DR. MAJIDI: Right.

QUESTION: -- and using other investigative techniques, you were able to say conclusively that all of these eight had come from USAMRIID?

DR. MAJIDI: That's right.

QUESTION: Okay.

QUESTION: Amber Dance from Nature. Can you explain to me why the flask would have contained a mixture of samples instead of just studying a clone?

DR. MAJIDI: Sure. That's -- you know, it has to do with the genesis of the material itself.

BACKGROUND OFFICIAL: It was a collection of many different individual runs taken from USAMRIID as well as Dugway Proving Ground.

BACKGROUND OFFICIAL: Yeah. And not to make things more confusing, but it's not uncommon to see multiple morphologies from a single culture that can arise independently from a single culture.

DR. MAJIDI: So this really was not -- the RMR 1029, the way to look at it is that a combination of multiple AMES culture mixed together in one container. So that's why the variations that you see, the genetic variations you see is so uniquely RMR 1029 because of those subsamples that were mixed together to form RMR 1029.

QUESTION: But why would Dr. Ivins have been working with a mixture?

DR. MAJIDI: That was the material that was used at USAMRIID and it was used for a number of different reasons, for research and development. That was the gold standard for USAMRIID.

DR. HASSELL: Yeah. The motivation was to get a large -- I'm trying to figure out words that won't confuse it with others, but, you know, a large container, if you will, of concentrated spores so that it could be used as -- for other testing, for challenges, for vaccine investigations, for example. So there was a driver to have one central grouping.

DR. MAJIDI: I'm going to ask Dr. Hassell to give you the story of the birth of RMR 1029. How's that?

QUESTION: Okay.

BACKGROUND OFFICIAL: RMR 1029 was a sample of pooled spores that were the product of many production runs, production runs that were from Dugway Proving Grounds as well as productions of spores that were produced within each sample. It was a large pool of spores because they required a sufficient amount to conduct their vaccine challenge experiments. Those spores were used to -- in aerosol experiments to make sure that the vaccines that they were developing were efficacious and for other research.

So it's not just one culture. It is -- it was actually flasks of pooled spores.

QUESTION: And is that the standard way to work with anthrax, to have a pool of different strains together, stored in a flask?

BACKGROUND OFFICIAL: Not a pool of different strains together. It was all the AMES strain but it was multiple --

QUESTION: Okay.

BACKGROUND OFFICIAL: -- production runs in order to have a sufficient quantity of spores in order to conduct their experiments.

QUESTION: Christine --

DR. MAJIDI: You look confused. Did you want to follow up on that?

QUESTION: I'm going to ask a clarification question on that. Christine Piggee with Analytical Chemistry, American Chemical Society.

Are you saying that the mixture of different mutations of the same strain in the flask was an artifact of production?

BACKGROUND OFFICIAL: Eventually could have been the case.

QUESTION: So it was a mixture of substrains?

BACKGROUND OFFICIAL: Correct. That's right. So -- but remember, the majority of the material is the wildtype AMES anthrax in that container and through various processes, you have the spore distinct mutation that appears in that flask.

Now, we don't know what the origin of the mutation is. We don't know if it started all in one batch or if it started in three of the combined batches or in two of the combined batches. We don't know. What we know is that RMR 1029 and its four genetic mutants within that container are uniquely RMR 1029. We will not find it in any of our others.

BACKGROUND OFFICIAL: Yeah. Understand that in the vaccine trials, this is a problem having a uniform challenge, you know, and so it was important to build a large enough stock so that you don't have to go back and reproduce it because when you do it, you might end up with a different substrain and hence not be able to compare the results of your experiments. So I think the presence of such a stock is not a surprise.

QUESTION: I'm sorry. If these mutations -- if some of these mutations arise automatically, I mean, I don't know how mutations arise because -- and it seems like that's an important question because, you know, the samples that you had have already undergone automatic mutation and the mutations that arose there, you know, happened to be the four mutations that you found in the flask.

BACKGROUND OFFICIAL: So we published several papers on this and in fact, you know, the theory of how mutations arise go back to the classic Gloria Dellbrick experiments and so at least the idea is, is that, experiments occur by random chance and

different regions of the genome will mutate at different rates and that's been shown in this whole syntheses and so just by stochastic processes, you will end up with mutations occurring and depending upon the rate, the intrinsic rate of those, they'll happen faster or slower and some regions mutate faster and some mutate slower, and understand that a population of cells that has, you know, 10 to the 9th cells has actually undergone almost 10 to the 9th generations of growth and so that's a very large number and if you have 10 the 12th, almost 10 the 12th generations of growth and so almost any mutation that you can imagine will arise during the growth of that.

The question is, is how soon does it arise and hence if it arises early, its frequency is going to be larger than if it arises late.

DR. MAJIDI: Let me see if I can ask you to just put a little more texture on that and talk about how stable these mutations are. Are these things just randomly appearing or are they stable over multiple generations, the particular four markers we saw?

BACKGROUND OFFICIAL: Well, the four markers that we saw have turned out to be stable over multiple generations and this was an assay that was -- this was something that was looked at very specifically because these mutations were deemed to be so potentially critical.

But if I might just make another more general comment to, I think, pick up on the discussion here, and it gets to the comments that Paul made. When I learned microbiology 30+ years now, it was clear that this notion of stochastic mutation was understood. We clearly didn't have genomic approaches to track these mutations, to measure them, but it's true not only in bacterial cultures, it's true in mammalian cell culture, and this has been well described in many, many publications, that repeated passage of bacterial cultures or mammalian cell lines tends to be associated with phenotypic changes.

This can be loss of virulence. It doesn't have to be, but when I was learning microbiology, I remember very distinctly what I learned was that in liquid culture, a large batch liquid culture should be considered whenever possible to be an end state in what you were doing and whenever possible you went back to low passage stocks as a starting point and from there, the next step was to plate out bacteria on a Petri dish or an appropriate solid substrate so you could actually look at to see what you had and colonies that appeared different would be, in most cases, apparent to the naked eye. They could have a different morphology. They could be pigmented or not pigmented, depending upon what you were working with, and it would be selection of a single colony that you would use to inoculate a flask.

It was considered bad technique to then take a liquid culture and use that for repeated inoculations because these stochastic mutations can accumulate and so if you think about it, if that's a natural process, but for all of the reasons why you don't in the process of vaccine development ideally want to be going back and generating multiple batches of material for development and testing, perhaps this puts all of this in a different context.

This is a trade-off between having enough material and some of the risks, if you will, or risks is perhaps not the best word, but some of the caveats that come with having to acquire that through the preparations of multi-flask use.

QUESTION: Thank you.

BACKGROUND OFFICIAL: I don't know if that helps.

QUESTION: That helps.

QUESTION: I'm going to follow up further on that. Rachel Erenberg from Science News.

And my understanding is both within the flask and within the Petri dish, you do get the stochastic mutations arising. How, in terms of doing sequencing, do you make sure -- how do you prevent actual growth of a dish because those mutations may arise in the course of the dish sitting in the cabinet? How do you know that the samples that you're sampling from, the time that you take the sample, are the same samples in terms of the genes, the genome snapshot that you're taking as they were when they arrived? How do you make sure they're the same?

BACKGROUND OFFICIAL: For essentially of the genome work that we have done, we have tried, I think in every case we have followed this protocol that I outlined, that when we have either prepared material ourselves or in the majority of cases they have provided material from outside collaborators, we have gone through this process of going back to low passage freezer stocks.

QUESTION: So what does that mean exactly?

BACKGROUND OFFICIAL: Clonal selection.

QUESTION: Sorry?

BACKGROUND OFFICIAL: Clonal selection.

BACKGROUND OFFICIAL: Clonal selection. It's really --

QUESTION: So you've got the addition of freezer that is highly --

BACKGROUND OFFICIAL: No, it's a slant or it's a frozen stock in a freezer that is -- it's going back to a stock that is not grown. Then you plate that material. It's plated out. A single colony is selected, grown as minimally as possible and for genome projects, you need a minimal amount of material. We're talking about a very different order of magnitude of material here for genome sequencing projects as compared to vaccine development efforts and that material is used for preparation of DNA, as a template for

genome sequencing projects.

But you raise an important point, that there is the possibility that during that process, additional stochastic mutations might arise, but I think what's important to bear in mind is that if they do, these are not random mutations that come and go. If mutations arise, certainly over the time frames that we're talking about in terms of genetic analysis that we carried out, they should persist.

We're not talking about large numbers of passages where minority representations in a population would be overgrown and the fact that these morphotypes that have been described were seen through repeated passage on plates could be identified through various assays that were developed gives us great confidence that in fact they were -- they represented stable components of these cultures. They were not -- they may have represented random mutations, but they were not random members of the population, if you will, that came and went at random.

DR. MAJIDI: Can I add one more item?

BACKGROUND OFFICIAL: I'd like to make a point of clarification. Many bacteria have phenotypic variations when you grow them as well as overnight in some bacteria. Anthrax is not known for that same practice. In fact, it's very homogeneous.

When one passes anthrax from one culture to another through passage, it's rare to experience any type of phenotypic variation or the mutations that we're talking about. In vaccine work, the Code of Federal Regulation actually limits for most bacteria the number of passages from your seed stocks to production of the vaccine to avoid this problem of mutation. Often that's as little as five to seven passages.

So in anthrax, it is very homogeneous. You don't see phenotypic variation and you rarely see mutations occurring with just a few passages. It was noteworthy that the anthrax powders that was in the letters had significant phenotypic variant numbers of phenotypic variants. This was unusual for anthrax and in particular unusual for AMES.

If one passes the 1981 AMES strain, the original AMES wildtype strain, one does not normally see mutations arising at a very high frequency. So it was noted that this was unusual in the anthrax powder.

QUESTION: Just for clarification because I've read different things. Did all eight samples have all four markers?

DR. MAJIDI: Yes.

BACKGROUND OFFICIAL: I just want to add one thing. One of the samples that was tested was actually DNA-extracted directly out of spores and those mutants. I think we should also clarify mutation is on the DNA. A mutant is a cell (inaudible). So this mutant was also found in the spore, in the DNA-extracted spore, meaning that those

mutants then arise during the culture process.

Another point. In genome sequencing, often having a subpopulation variable that -- often talking less than one percent, it's never really a problem because when we sequence a genome, every bit of DNA is sequenced several times and from different molecules and those mutants actually don't even make it into the consensus sequence which was coming from the major component which is the wildtype.

QUESTION: And so what TIGR did was it sequenced the entire genomes of all the variants found in the sample?

BACKGROUND OFFICIAL: So we were handed over DNA. The DNA was compared with some of it in the lab. We sequenced for three of the letters, the wildtype genome, and two of the wildtype for each of the three letters, and then for only two letters we sequenced another different morphotype and only one, the fourth, the wildtype.

So in total, there was about 12 genomes. The wildtype was completely 100 percent identical in every way, every single one of the (inaudible), including the plasma, it was identical to the strain that we -- the genome that we pulled the AMES strain which is actually (inaudible) and the other morphotype had differences, unique differences and each of the morphotypes which were identical morphologically in each of the letters had similar differences on them in the genome sequence.

The sequencing that was performed was Sanger sequencing which at the time was routine and the most accepted and validated method. The advantage of Sanger is that we start from a clone and sequencing from a clone, you can generate some very good sampling and the advantage of having chromatogram which comes from the sequencing also gives you a very high confidence in the quality of the sequence and each base pair was sequenced an average of about 12 times.

So we were highly confident that the mutations were true and we were also able to, like Claire said, to eliminate mutations which come from the draft genome which were the quality based on the chromatogram was not high enough and Paul can confirm that this approach is actually very popular to pick only the right and true mutation.

BACKGROUND OFFICIAL: Yeah. I was going to pat Jacques on the back for this quality pipeline that TIGR developed because, you know, nobody had ever really sequenced things that were so closely related before and early on, we did in fact pursue some sequencing errors but very, very quickly in the investigation, it was learned that the quality scores of each base from Porton and TIGR was able then to prioritize real differences and really change the way that we did the laboratory work based upon their bioinformatic skill.

QUESTION: Could you just clarify how many strains or substrains were actually sequenced versus how many were just checked for those four mutations?

BACKGROUND OFFICIAL: 12 were sequenced.

QUESTION: And these 12 from the letters were sequenced and then you checked all the samples that were submitted to the FBI for those mutations?

BACKGROUND OFFICIAL: Yes. Correction. Only -- out of the 12 that were sequenced, four genetic mutations were chosen to develop assays to screen.

QUESTION: Okay. Those weren't necessarily all the mutations, they were just the ones you used?

BACKGROUND OFFICIAL: Some of the isolates that were -- that came out of the letters were also checked for those mutations.

QUESTION: Okay.

QUESTION: Could you tell us a little bit about the assays themselves? The assays were developed after you had fully done the sequencing for the 12 variants and discovered four mutations.

BACKGROUND OFFICIAL: It's not 12 variants. So each letter had a set of one wildtype and a certain number of variants.

QUESTION: So in all, there were 12?

BACKGROUND OFFICIAL: Four different type of variants were sequenced and several times, depending on which letter, and three wildtype isolates were sequenced from three of the letters.

QUESTION: So there were 12 letters?

BACKGROUND OFFICIAL: No, four letters.

QUESTION: Four letters. Four letters. From each letter, you sequenced, depending on, you just said, wildtype and subtype. Can you just repeat the samples?

QUESTION: So could you do letter number 1, what was in it, letter number 2, what was in it?

BACKGROUND OFFICIAL: So as I recall, in letter number 1, we had wildtype, what USAMRIID

BACKGROUND OFFICIAL: It's a phenotypic, yes.

QUESTION: So you don't -- okay.

BACKGROUND OFFICIAL: And letter number 2, we had a wildtype, Type A, Type

B. Letter number 3, we had a wildtype, Type A, B, and what we call D Morphotype, and in letter number 4, I think we had -- none of them were sequenced but they were checked. The mutation that went behind the other letters were checked for these and the isolates that were found in that letter, which I think is the Daschle letter.

QUESTION: So in all, there were four variants that were completely sequenced?

DR. MAJIDI: Let me ask one of our guys to kind of go over this procedure one more time.

BACKGROUND OFFICIAL: As I said, when we examined the anthrax for a pattern by culture, many variants were found and Dr. Keim prepared DNA for a lot of those which was sent to TIGR and they sequenced many of those, but we really -- the FBI decided to focus on four of the mutants because of the types of mutations that were found. We felt that those would be the most pertinent forensic signatures to exploit in our tests of all the other evidence.

So we did not use every mutation that was found and discovered by TIGR when we screened the evidence.

QUESTION: Right. But how many different strains were sequenced fully in order to determine what mutations you would use to assay the 1,000 samples?

BACKGROUND OFFICIAL: 12.

QUESTION: Right.

BACKGROUND OFFICIAL: 12 different phenotypic variants were sequenced.

QUESTION: Right.

BACKGROUND OFFICIAL: We chose only four of those to develop assays.

QUESTION: So they're all eight a strain of them?

QUESTION: Right.

BACKGROUND OFFICIAL: They were all confirmed to be AMES, but there were phenotypic variances and 12 of them were sequenced and four were chosen to further develop assays.

QUESTION: Right. And that brings me to the question of how the assays were developed.

BACKGROUND OFFICIAL: Well, the assays were developed as, Dr. Majidi's opening statement said, by a consortium of contract laboratories that the FBI used. We

felt that we had to get this done fairly rapidly. To have only the FBI laboratory develop the assays would have been impractical. So we reached out to the experts in the biodefense community and had several laboratories develop the assays for us.

QUESTION: Can you just detail what you mean by "assay" or are you just using PCR and sequencing those particular spots or what?

BACKGROUND OFFICIAL: Well, the sequencing information told us what the mutation was and then they were PCR-based assays to detect that specific mutation within a very overwhelming background of normal wildtype --

QUESTION: Are these single point mutations? How -- what kind of -- how much of a structure was used?

BACKGROUND OFFICIAL: We purposely chose not to exploit single point mutations because of the frequency of occurrence in cultures and we relied on the four mutations that we chose were much more significant.

QUESTION: Like 10 base pairs?

QUESTION: Deletions, additions?

BACKGROUND OFFICIAL: They were insertions and deletions.

QUESTION: And just to get back to Rachel's question, can you give us a sense of how many that was, you know? Was it like a few dozen base pair differences or --

BACKGROUND OFFICIAL: Can you hold on?

DR. MAJIDI: So this actually falls in the category that I'm -- please respect the publication process.

QUESTION: Oh, okay.

DR. MAJIDI: So this actually may very well end up in one of your journals and you definitely don't want to have this predetermined before we put that through the peer review process.

QUESTION: Sure. We want to write about it again.

DR. MAJIDI: All right.

BACKGROUND OFFICIAL: Suffice it to say that they were significant.

QUESTION: In a lot of the press, we've seen over and over that there new methods developed very recently that broke this case. Can you be specific about what these, quote

unquote, new methods are?

DR. HASSELL: Yeah. We tried to touch on it a few times. The more precise way of putting this is that they were established techniques and methods, but they were applied in new ways to basically become the foundation for forensic microbiology.

QUESTION: I note that this question that I'm going to ask might sort of get away from the science a little bit and, you know, but it is intertwined with the science.

So you say that through other investigative processes, you were able to determine that the eight samples that contained all the four mutations came from USAMRIID, --

BACKGROUND OFFICIAL: Yes.

QUESTION: -- and when I asked you about that, you said, well, you know, a simple look at the notebooks might have been one way of doing that. Shipment records.

BACKGROUND OFFICIAL: Sure.

QUESTION: We're not going to get into that, but that's what we did. But that also raises the question as to other labs, you know, that were sent the USAMRIID samples --

BACKGROUND OFFICIAL: Sure.

QUESTION: -- and other labs that could have been the source of the attacks.

DR. MAJIDI: Well, I'll conclude with just saying that if you layer various tiers of investigation, right, systematically you work through everyone who was at some point in contact with a sample and we arrived at a particular location with a particular suspect, we narrowed it down through the investigative process, so that the fact that there were eight samples out there, that's a very, very good point.

What I can tell you from the investigative point of view, we have investigated everyone who was related to any AMES strain and narrowed the search down, narrowed our focus down to AMES strain, plus those particular variants of interest.

BACKGROUND OFFICIAL: And particularly looking at those who received samples from RMR 1029. Those were investigated very heavily.

DR. MAJIDI: So again for the investigation, what I'm asking you is not to second-guess our investigative approach. This is something that we've been doing for a hundred years and we're pretty good at it. It's science development. The application for this case is relatively unique and that's what I'm really here to share with you.

QUESTION: I'd like to know something more about the phenotypic differences among the four, and can you say something about it, as to whether those phenotypic differences

are logical based on what you know about the mutations of the genetic level?

BACKGROUND OFFICIAL: The mutations or the phenotypic variation that was seen were physical characteristics, such as the difference, slight difference in color of the colonies, slight difference in texture of the colonies and morphology of the colonies.

QUESTION: Can you be specific, like slightly more pink or --

DR. MAJIDI: How do I know the color pink to me is the color pink to you? So just accept the fact that morphology was sufficiently different that we were able to pick them up.

QUESTION: Okay.

DR. MAJIDI: Some more fuzzy than others.

QUESTION: So in anthrax in particular, the kind of morphological differences that arise are texture. I mean, what is the color range for anthrax?

BACKGROUND OFFICIAL: This is a fairly standard microbiologic approach and speaking of culture, some colonies will be smooth with completely irregular circumference, others will be irregular zygotes, as we refer to them, and this goes back to Robert Koch at Reichs in the early microbiology, fairly standard, and these colony types can be very selective picks and carry and continue to be smooth but occasionally smooth colony and will throw off a number of colonies. So it's a kind of -- it's a standard microbiology approach in characterizing phenotypes, the morphological colonial phenotypes.

QUESTION: I want to go back to something you mentioned before about what I and I imagine all my colleagues are hearing from scientists is we haven't seen enough evidence, we want to see the rest of the evidence, and I understand you're going to publish it.

Could you talk about whether it's been submitted, when it might come out?

DR. HASSELL: Because like I say, some of it is already out. It just it wasn't obvious from the publication it was related to this case. We were going to provide that list. We hoped to do it today, but just because of privacy concerns, we needed to make sure we contacted all the co-authors. So we're ready to release that very soon.

We've identified a number of papers that we feel will come out of this, both from within the FBI laboratory, external to the laboratory, and then some in collaboration between the two. So we've identified on the order of, you know, at least putting on putative titles, of course, we haven't gone back through, but, you know, a dozen or more that we think will come out of this and so what we're trying to do is line everything up to get those rolling. We want to get that going as quickly as possible.

Again, I mean, we really respect the peer review process and we really want to try to use that to its full advantage to -- as another layer of validation to all the science that was done.

QUESTION: I want to go to the additives and I understand that the Armed Forces Institute of Pathology in 2001 had done the sort of first analysis of the spores and had determined that silica had been added to the spores that were found in the letters sent to Senator Daschle.

However, you were saying that no additives were used and then there's also this intriguing text about the silicon signature in the affidavit.

If you would reconcile these things for me?

DR. HASSELL: Well, as the work progressed on this and more and more sophisticated techniques were brought to bear on just the silicon question, just to take that as an example, we brought more people in to advise on this, to look at it in much more detail.

So I'm going to ask Dr. Michael if he might comment on it and then we can bring it back and then talk about some others, if we need to.

BACKGROUND OFFICIAL: Yeah. It was -- when we got the evidence the first thing we did was put them in assay and take a look at them and we indeed did see signature of silicon and oxygen. What we didn't see was any sort of nanoparticles on the outside of the spores. They just looked like regular spores to the experts that were advising us.

So at that point, we knew there was silica or silicon and oxygen within the spores, but we weren't sure and so we used some more sophisticated techniques. I've been working at Sandia for quite awhile now with hyper-spectral imaging and particularly for x-ray imaging on the scan (inaudible).

Whenever you hit a sample with electron-generated x-rays that are characteristic of (inaudible) and so we use hyper-spectral imaging so that every pixel and image we would get would have a full analytical signature of silica.

We then take this data and put it through some statistical analysis that then shows us how the various signatures are related. In fact, we found that when we took the spores and put them in the transmission electron microscope, we would run sections of spores, just take them three to the power, we would get the silica signature, but now they were sort of located on the outside of the spores. It wasn't until we actually had ultra microtome sections in the transmission electron microscope and the scanning transmission electron microscope where we could localize the silicon and the oxygen signal to the spore coat and not to the exosporium. So it was on the inside of the spore and not on the outside of the spore.

QUESTION: Is that common? Does the anthrax usually have some kind of spore --

DR. MAJIDI: You know, this is a -- if you look, ultimately what we're saying is that that is not a postproduction additive to make the anthrax more dispersible. That's what the whole concept or methodology of weaponization comes from, is to weaponize. That's really -- that's an ambiguous word, but what people mean by weaponize is that postproduction of the spores was silica added to it to make it more dispersible.

QUESTION: Does the anthrax do that?

BACKGROUND OFFICIAL: There are reports in the literature and Professor (inaudible) who indicated the same sort of information.

QUESTION: And I'd like to clarify. Are we talking about the element silicon or are we talking about silicon dioxide, otherwise known as silica?

BACKGROUND OFFICIAL: We found -- we don't know the stoichiometry of what we found. We know that there was S, silicon and oxygen collocated. We don't know the relative mixtures of those, so we can't say it's SiO_2 or what.

DR. HASSELL: There's so many environmental sources.

BACKGROUND OFFICIAL: We actually did find it in vegetative cells that were in the sporulation process. We found those spores within the cells and still could not determine it, and I am suspicious of the preparation techniques with the sections. We tried different techniques. We still ended up with the same results.

DR. MAJIDI: So one last time. No additive was added to the sample to make it more dispersible.

QUESTION: Well, now it becomes believable because now there's, you know, -- you're presenting the scientific process by which you arrived at that conclusion.

DR. MAJIDI: Right.

QUESTION: You know, what we had in the affidavit before --

DR. MAJIDI: That's why we have an open record here, so you can have all of this information at your disposal.

BACKGROUND OFFICIAL: and it should be noted that there's a lot of literature that shows that the genus *Bacillus* mineralizes and incorporates not just silica but metals and lots of minerals and that there's at least 30 years of literature documenting this.

DR. MAJIDI: Did you have a question?

QUESTION: I did. I wanted to get back to the peer review thing we were discussing. I

know that Dr. Fraser-Liggett has told me that the different labs didn't necessarily know what each other was working on.

Did you do any sort of peer review during the investigation, having one independent scientist check another's work?

DR. MAJIDI: Many.

BACKGROUND OFFICIAL: Dr. Colwell is a prime example of some of the people that we consulted and routinely told our science, the science that we were doing, vetted it, draft the scientific community, as well as our scientific working groups at the FBI, sponsors and almost all the forensic disciplines, and also, more specifically for the case and for the investigators, we invited scientists in and conducted what we call our red team review and presented more specifics of the science to these people under a non-disclosure agreement and asked them what they thought, which also led to additional experiments to validate the work.

DR. HASSELL: So another way of phrasing that is we vetted both the approach, the process, like I mentioned earlier, as well as the results.

BACKGROUND OFFICIAL: I should also add that perhaps one of the benefits from this tragedy with respect to the victims is that a good deal of investment has been made in microbial systematics and affiliated ecology and there's a whole set of ancillary studies on other pathogens of *Bacillus*, cholera, that will be appearing that confirm in parallel ways the findings that you are hearing presented today.

QUESTION: We know that with this kind of work, a lab is establishing that something is the same as something else is a matter of statistics.

Can you talk about just how many total samples you're comparing these strains against and what statistics you brought to bear on them?

BACKGROUND OFFICIAL: There were over a thousand samples of *Bacillus anthracis* AMES that was collected or identified by the investigators, that were placed into the repository that we screened, and I think it's significant to note that there were only eight of those over 1,000 samples that had all four of the genetic mutations that were present in the anthrax letters.

DR. MAJIDI: And just to stay with my non-discussion on the investigative approach, it's really critical to point out that all of those eight were linked back to a single origin.

QUESTION: Dr. Ravel, we've heard a lot of praise for your quality control process. Could you tell us a little more, a bit more about this pipeline that you've developed?

BACKGROUND OFFICIAL: The pipeline is -- takes closed genome, in this case, with AMES ancestor, the strain that was isolated in 1981. So we have a very high-quality

genome sequence for this strain.

The genome sequence, like in the Gene Bank, doesn't come to us like the consensus sequence. We have access to the consensus, but all the reads, all the sequences that makes up the consensus sequence. So for the genome that we sequence, we have access to the same thing, the consensus sequence.

So we put together a pipeline that compared the consensus sequence and for this, we incorporated and modified the program that's called MUMMERT that was headed up by a team at TIGR back in 2000 or so and it's still being improved at this time, and that allowed us to identify any polymorphism between the two genome sequence as well as insertion and deletion.

Now, once those polymorphisms are identified, then the pipeline goes back to both the reference sequence as well as the sequence that we are analyzing and collects the information of all the quality and the identity of the base pair that made the consensus not only for that point but about 40 base pairs around that point or insertion/deletion and then we analyze each read that made up that consensus and look at the quality of each of those reads and we have a set of criteria that allowed us to validate and say yes, this is a true polymorphism or no, it's not a true polymorphism.

The criteria have -- are quite stringent. We have a different level of modifying those polymorphisms. The only one that we're using in this analysis were what we call high-quality, high-coverage polymorphism where each read is -- the quality of each of the read is 30, meaning that you have one chance in a thousand that it's not the right base pair in this position in one single read. So it had to be 30, at least 30 on each of the reads that made up the consensus sequence and the coverage needs to be at least three X. So each base pair that we looked at had to be sequenced at least three times, and at the end, we have a list that we provided and it includes all this, the coverage, the quality, and the position.

QUESTION: How does this compare to an average lab doing a sequence and putting it up on GenBank? Can you give me an idea of the scale?

BACKGROUND OFFICIAL: Well, in an average lab, what people, at least in GenBank, we don't have access to that, you know, information that makes that -- underlies the consensus sequence. So for us, you know, doing the sequencing and having access to that quality is essential. The information should be published as well.

QUESTION: Can we just go back to the statistics real quickly? Having these four mutations against a thousand, you know, and X number of samples against -- in -- within this bigger sample, what are the odds of that in terms of the P value or, you know, this is something that if we had these -- if we had the paper, this is what we'd ask the outside scientists, but we don't have these tests?

BACKGROUND OFFICIAL: Well, it's a difficult statistical analysis to perform,

although it sounds fairly simple. If there were 1,070 some odd samples and only eight had all of the mutations that we were looking for, that sounds like it should be relatively simple.

But you have to understand that the combined investigative approach behind those samples. There's additional data, for instance, the BA samples that did test positive investigatively have all been linked to one sample. So they are (inaudible) skew your data somewhat because there is a linkage, not a genetic linkage, but a source linkage in that data, and throughout the repository there are numerous source linkages as scientists passed strains from one to another. So it's very difficult for us to come up with a statistical figure that would give you an easy feeling about the result of the data.

DR. HASSELL: We should point out that the analyses, those assays that were run on those 1,000-plus samples, those were done blind. There was no preconception about the origin of any of those.

QUESTION: Can I ask a similar question but in a very different way. Did any other samples have three of those markers but not four; or two of those markers but not the four?

DR. HASSELL: Yes, there were some that had --

BACKGROUND OFFICIAL: There were none that had three and there were a few that had two and there were some that had one. But there were small amounts of each.

QUESTION: These 1,000 samples, were they from labs across the country or across the globe?

DR. HASSELL: There were some international samples.

QUESTION: But primarily U.S. samples?

DR. HASSELL: The majority of them.

DR. MAJIDI: Remember the AMES strain was really a U.S.-driven strain so it's not unusual to have the majority of them from the U.S.

QUESTION: What made the FBI release the scientists from the non-disclosure agreement today?

DR. MAJIDI: Well, the plethora of misinformation, that's clearly one of them.

QUESTION: So it works. [Laughter.]

DR. MAJIDI: The reality of it was we felt we were at the point in this case that it was prudent for the American public to be aware of our investigation. This is incredibly

unusual for the Department of Justice and the FBI to sit down and talk about a case at this juncture. Unfortunately, there was a sequence of events that led us to where we are today. It's not a place that we want to be. It's not a place that the Justice Department wants to be. I think all of us, including you, should feel as we would have liked to see this through its natural conclusion, which would have been a court decision of our findings, as well as the rest of the information we have. Having said that, we needed to have a closure for victims' family as well as the public interest.

BACKGROUND OFFICIAL: May I add also that this is the birth of an entirely new discipline that embodies forensic microbiology, proves its worth; its value; and it's another tool for future occasion.

DR. HASSELL: And I'd even go beyond that. There's public health aspects of this, too, by being able to respond more quickly by using better tools and more validated processes. I think several people on -- I know Background Official's touched on this, as well as of our discussions --

BACKGROUND OFFICIAL: I mean, we've had the advantage of having a great deal of money and public health has not had the same level of funding that we've had; but the technology's becoming much more affordable and so it's easy to predict that, in fact, food-borne diseases and other types of infectious diseases will be investigated in a similar fashion in the future; in the near future, actually.

QUESTION: Do you anticipate closing this investigation anytime soon?

DR. MAJIDI: Well, soon is a relative term so I don't want to give you a particular date, but we are working through the last portions of this investigation and ultimately it will come to a conclusion and the case will be closed some time in the future.

QUESTION: I just had one last question about the timeline. Around when were you skirting that the Anthrax had come from USAMRIID?

BACKGROUND OFFICIAL: Well, as Mr. Majidi said there are some aspects to the investigative portion of this that are not appropriate in this forum. But the science behind all of the markers was complete some time in early 2007. So it was a long process which began in 2002 and was not concluded until 2007.

QUESTION: Can you talk a little more about this Red Team process and how many meetings were held? I know you can't tell us who was there, but for example, what timeframe, with what frequency?

MR. MAJIDI: The Red Team happened -- let me just tell you various advisory boards and various data validation happened throughout this case, at multiple stages. I really don't want to provide you with any specifics of who did what, who did validate what; but (inaudible) DNA of this process itself. As we went through every step we made sure that the data was validated. Remembering that we have been in the forensic business for a

very long time we understand the requirement that it takes to take a data or a piece of evidence to court. We have all of that in the back of our mind. Every piece of experiment or every piece of tool that we apply to this problem was with the mindset that it must be fully vetted and validated before we could accept the results.

DR. HASSELL: There are some aspects of this, too, that we have to be careful of for countermeasures. We need to make sure we don't give the bad guys every single bit of our whole strategy so as to be used against us in the future. So I say we're going to publish this and disclose as much as we can. I'm sure there will still be individuals out there who will think we're being evasive and I have to say, it's just what we have to do for national security.

BACKGROUND OFFICIAL: But the scientific working groups are a long tradition at the Bureau, and in fact, they were involved in this process all along and a number of us are on those; and that's public information.

QUESTION: So can we tell our readers that until something comes out in peer review publication about the information that you're giving us is all that they're going to get?

DR. MAJIDI: No, there are already publications out there. What we don't have right now is the list of publications that are out. More than likely we're trying to assemble a website to list everything that's associated with the science and technology part, including the complications and ultimately what we put out after we have every issue worked out; the name of some of the individuals that were really critical to our success. So there is a good number of publications that are already out. In fact, Claire already mentioned her publications and so forth, and clearly you can look those up. It's really not too hard and see what was published in the past seven years and see how it relates to the potential of this case and where did it come from. But we don't actually have the information out for you as well as any future publications.

BACKGROUND OFFICIAL: There already have been scientific presentations made by people like BACKGROUND OFFICIAL at the Bacillus conferences, the American Society for Microbiology, that actually showed pictures of the mutants themselves, the colonies and what their morphology looked like; and the approach to sequencing those mutants and determining mutations of those. There have been snippets of investigative science that has been released already in the form of presentations at symposia as well as publications.

DR. MAJIDI: I think everyone who is involved, at least in this panel, you can see their work has been presented at scientific venues; not in light of the Anthrax case. Are we getting the point that none of the reporters have any questions? [Laughter.]

QUESTION: There seems to be some issue with perhaps running out of sample at some point. Can you talk about what step was the most laborious or took the most amount of sample for analysis?

BACKGROUND OFFICIAL: We had limited sample from some of the letters as you can imagine. They went through the mail. I think we had -- some of the letters we had sufficient samples, certainly to conduct all of the tests and examinations that we needed so I don't think we were in jeopardy of running out of sample.

QUESTION: No, I mean, I'm just saying out of all these different methods you used which one you need the most sample to run.

DR. HASSELL: I know we have that information I just don't have it off the top of my head. But the reason that we have that is because we had to go through that in the initial planning process because we had to finite them out. You know, if we had a kilogram then we knew we could probably do a lot more with that but we only had a small amount so we actually -- a lot of the working groups went through, assuming you had "x" amount, and then figured out how much you would need; and that's how we through out some techniques. It just wasn't feasible to use them.

QUESTION: Can you talk about which techniques you threw out?

DR. HASSELL: I don't remember specifically. There really weren't that many because I mean, I'm more on the analytical chemistry side than with the microbiology side of it just because my own background is what I participated in personally. There wasn't that much that we had to throw out really because the techniques have gotten so much better, you know, microsampling, it would be worth -- if we could document that process it might be useful.

BACKGROUND OFFICIAL: It's more not a question of techniques being thrown out; all the scientific techniques that were applied to the investigation actually provided fruitful data. The question is whether that data was probative to the investigation.

DR. MAJIDI: We have lots of information that really just didn't get us anywhere.

QUESTION: I've read a little bit of that there was some sort of contamination of *Bacillus Subtilis* in these samples. Is that true and is there any significance to that?

DR. HASSELL: Like Dr. Majidi mentioned in the beginning, there were differences between the letters themselves. There were, essentially he showed that there were two batches that were made from this and one of those batches appeared to have been contaminated with *Subtilis*.

QUESTION: Is it typical to see or is it unusual to see *Subtilis* contamination with Anthrax?

BACKGROUND OFFICIAL: It is possible always to contaminate potentially a liquid culture or if you're growing large quantities of an organism where you're growing lawns of a bacteria on a Petri dish it could be difficult to see other members of the bacillus species because they would be occluded by and look very similar to the organism that you

are culturing. So it appears at some point a low level of bacillus contamination occurred in the preparation of the material and that was detectable and was able to be isolated and characterized.

QUESTION: Is there any sense of where that might have come from along the process?

DR. HASSELL: Well, it doesn't exactly answer -- well, I'll get to that but one other point is that the Subtilis contamination was also in the samples that also had which looked like a rougher preparation, so there was more darker color. They were rougher.

QUESTION: That was the first one.

DR. HASSELL: The particle sizes and other things wasn't as refined a sample. So it's consistent. If we found that in the other ones it might have raised more question but it just looked like one was just a rougher preparation than the other and that rougher one had the Subtilis.

BACKGROUND OFFICIAL: The B Subtilis is a common environmental organism and so incomplete sterilization or perhaps some sort of settling in media would enable it to be co-cultivated.

QUESTION: I understand that. If Ivins was such a good microbiologist I'm wondering how likely he would be to contaminate his sample to where it might come from.

DR. BURANS: I consider myself a good microbiologist and I've contaminated cultures in my life. [Laughter.]

QUESTION: In the early days of the coverage of this case a lot was made of the granularity, the fineness of the spores, and I wonder if any of the scientific work that was done addressed that question?

DR. MAJIDI: We looked at a lot of different type of microscopy. We looked at the distribution, the science and all that stuff and, you know, it was basically we narrowed it down to two preps, you know, one rougher, one not so rough.

BACKGROUND OFFICIAL: I caution anyone to making too much out of the particle size or granularity. We don't know what the material looked like before it went through the high energy of the mail sorting process. So I don't believe that much can be concluded if we don't know what the material looked like before it went through the barcode.

DR. MAJIDI: We found agglomerates. Not every spore was sitting happily as a spore form waiting to be dispersed. Like any prep, you would see agglomerates. You could see single spores. You'd see a mixture. So it wasn't unique enough to say, boy, every spore was painstakingly thought of individually.

QUESTION: A while back, there was a lot made of this borrowed lyophilizer. Can you just talk about the difference between the spores you took out of the letters versus the spores that would have been sitting in the flask, and what process they might have gone through to get that way?

DR. MAJIDI: Which flask are you talking about?

QUESTION: So the flask that allegedly Ivins started with, there were spores in there.

DR. MAJIDI: Right.

QUESTION: Were those spores, I mean, different from the ones in the letters? And how so?

DR. HASSELL: They were regrown. I mean, they weren't just pulled out of that flask of 1029 and then dried and used. There were chemical differences. There were some chemical constituents in the flask that were not present on any of the samples. So we do know that there was regrowth there. Post-processing, we just really can't speculate without treading too far on the other side of things. I mean, he did have access to some post-processing equipment, but that doesn't necessarily say that was used.

DR. MAJIDI: But you all know as well as I do that you can dry material in a number of different ways. And it's not -- we don't know what the methodology was, but you know better than I do you can dry stuff in a half a dozen different ways. A lyophilizer would be one of them.

QUESTION: At any point, did you find matching *Bacillus anthracis* samples in any place besides the laboratory during the investigation?

DR. MAJIDI: What we found was in RMR 1029, and then the repository, and the letters. That's where we found the stuff.

QUESTION: Dr. Colwell, could you tell us how much money NSF actually gave out for this work?

BACKGROUND OFFICIAL: The small grants for exploratory research at that time were, I think, \$100,000. And they can be made -- the awards can be made very quickly. They can be internally reviewed, or at least they could be internally reviewed. And so we were able to respond very quickly. And I would add that at the same time, we made similar grants to engineers at Berkeley and in Florida to go to the Twin Towers site, especially one engineer from Florida who had developed a robotic mechanism for photography, and was able to use that to search for victims. So these grants could be made, and we were glad that we could do that. We could respond very quickly.

MR. KORTAN: Dr. Majidi, we can take one more question.

DR. MAJIDI: We'll take one more robust question.

BACKGROUND OFFICIAL: Just one quick thing.

DR. MAJIDI: Sure.

BACKGROUND OFFICIAL: The sequencing effort was a combination of both NSF and NIAID and NIH. They participated financially.

BACKGROUND OFFICIAL: I'd like to emphasize that ongoing support was through other agencies -- NSF, NIH, Department of Energy. Also, some funding for some of the sequencing work was provided by Agriculture. It was a consortium of agencies working together subsequent to the initial sequence award.

QUESTION: You know, one other thing that has appeared in the coverage is that investigators tried to reproduce or remake the powder that was used in the mailings, and had difficulty in doing so or wasn't able to do so. Is that all true?

DR. MAJIDI: We just went through that. The two different preps were different. If I make soup at home two different times, they are not going to taste the same. So the fact that we can't exactly duplicate a single particular prep is not unusual in any realm. And again, I'm going to ask Jim just to put a little texture on what I'm saying here.

BACKGROUND OFFICIAL: Yes. There's so many variations in biological systems that occur, just variations in humidity, temperature, qualities of your media, that could affect the ultimate end product. And how you actually handle and harvest the material, whether you grow it in broth or grow it on agar plates, how you resuspend the growth, how you allow the material to sporulate over time -- in some aspects, this is an art form. And because we're dealing with biological systems, there are always components of variation that are introduced into the process.

DR. MAJIDI: All right, folks. Thank you very much. I appreciate your time. And hopefully we've answered all the pertinent questions you guys had with respect to this case. Go out and dispel any --

(Laughter.)

END 11:37 a.m.