

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: "Old" formaldehyde experiments
Date: Wednesday, September 12, 2001 9:47:56 AM

(b) (6)

Here is the information you requested on the Covance study:

Five years ago we made rPA vaccine/Alhydrogel with and without formaldehyde added. We tested the vaccines after various periods of time of storage and noted (in guinea pigs) that the presence of formaldehyde appeared to boost potency of the vaccine. It was unknown whether the boost in potency was due to stabilization of the protein, or to an adjuvant effect. (Formaldehyde itself causes local inflammation which would draw APCs and other cell types to the site.) The vaccine is now 5 years old since it was formulated, and we wished to see (in the rabbit model) if there is any difference in potency between the 2 vaccines. (The rabbit model is preferred over the guinea pig model in tests of anthrax vaccine efficacy.)

Twenty-four New Zealand white rabbits (12 of each gender) were immunized with 0.5-ml intramuscular doses of vaccine containing 50 micrograms rPA, Alhydrogel (0.5 mg aluminum) and PBS (with formaldehyde, 0.02%).

Twenty-four New Zealand white rabbits (12 of each gender) were immunized with 0.5-ml intramuscular doses of vaccine containing 50 micrograms rPA, Alhydrogel (0.5 mg aluminum) and PBS (without formaldehyde).

Four rabbits (2 of each gender) will be controls receiving Alhydrogel and PBS.

Rabbits will be bled at weeks 2 and 4 for anti-PA antibody titers. They will be challenged subcutaneously with virulent anthrax spores 6 weeks after immunization and monitored for survival.

This experiment will demonstrate whether the presence of formaldehyde in an rPA/Alhydrogel vaccine increases or preserves potency.

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: CRM Task Order 0086
Date: Wednesday, September 12, 2001 1:26:29 PM

I approve.
- Bruce Ivins

>-----Original Message-----

>From: (b) (6)
>Sent: Wednesday, September 12, 2001 1:26 PM
>To: Ivins, Bruce E Dr USAMRIID
>Cc: (b) (6)
>Subject: CRM Task Order 0086

>

>Dr. Ivins;

>

>I have received invoice no. 11 for (b) (6) for the month of August for \$5,442.33. Please email reply to all your approval. Any questions, please call.

>

>Thanks 9/12/01

>

(b) (6)

[Redacted signature block]

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Protocol modifications
Date: Friday, September 14, 2001 10:32:09 AM

(b) - We need to wait until (b) (6) finishes his 2-dose study to decide what dose of rPA to use. If it turns out the dose is 10 micrograms -which is a good guess, will you have enough rPA for 2 shots, 30 rabbits of each isoform and 30 rabbits with rPA?

Responses to (b) (6)

1. It is OK with me if we go to the larger number of rabbits.
2. OK.
3. This does not need to be a GLP study. This is still a tech-base study to tell us whether there are differences (as far as vaccinating ability) between the two isoforms. If it needs to be done GLP, then Battelle should do it.

- Bruce

>-----Original Message-----

>From: (b) (6)
>Sent: Tuesday, September 04, 2001 8:36 AM
>To: (b) (6)

Ivins, Bruce E Dr USAMRIID

>Subject: FW: Protocol modifications

>

>Re: animal protocol to study the contribution of rPA isoforms to protection against a lethal anthrax infection in rabbits. (b) (6) suggests we do 30 rabbits per group; see below. The change would result in 94 rabbits instead of 52 (\$\$\$?). Please advise if we wish to accept his recommendations so I can make the changes. (Bruce had originally suggested 16 per group). Thanks in advance.

(b) (6)
> << File: Rabbit isoform protocol23Aug.doc >>

>

>-----Original Message-----

>From: (b) (6)
>Sent: Friday, August 31, 2001 3:30 PM
>To: (b) (6)
>Subject: Protocol modifications

>

>

> The isoform protocol is suggested to undergo the following statistical revisions. There are three reasons

> for the revisions:

>

> 1. This study is approaching the final formulation of the rPA anthrax vaccine candidate and thus must

> adhere to stricter criteria.

>

> 2. The stricter criteria involve the concept of "equivalency" testing. This means that we seek to reject

> the hypothesis that the two isoforms are NOT equivalent with respect to survival and immune response

> as measured by ELISA and TNA. Rejection of the hypothesis establishes the statistical evidence that the

- > isoforms are equivalent. This requires larger sample sizes than a test of the hypothesis that the two
- > isoforms are not different (a subtle difference), which is an early developmental hypothesis.
- >
- > 3. Should this not be a strict GLP study due to the need for FDA approval of the decision that will
- > arise from it? We do not wish to have to repeat the study in the future under GLP standards.
- >
- > Revisions suggested:
- >
- > Page 3 replace objective with: "The hypothesis is that the two isoforms are NOT equivalent in survival rates
- > or immune response as measured by either ELISA and TNA. Equivalency is defined as a difference
- > in survival rates of no more than 20%, and a ratio of geometric mean ELISA titers and TNA titers of
- > no more than 4-fold respectively. The hypothesis will be tested at the 95% confidence level (2-tailed). Rejection
- > of the hypothesis establishes the equivalency of the survival and immune response of the two isoforms.
- > Failure to reject any of the hypothesis (survival or immune response) fails to establish the equivalency
- > of the two isoforms. Sex-specific tests will be done."
- >
- > Page 11 replace Data Analysis with: "Sex-specific survival rates will be tested for equivalence using the method
- > of Farrington and Manning (ref). ELISA and TNA titers will be transformed to log10 and analyzed for
- > sex-specific equivalency based on the 95% confidence intervals (2-tailed) around the ratio of geometric mean titers.
- > Further analysis of survival correlations with sex, titers and isoform will use standard logistic regression.
- > Also, further analysis of titers will use multiple regression to test for sex differences and to test for
- > isoform differences. All tests will be at the 95% confidence level (2-tailed). All data will be automated
- > and verified prior to analysis. Statistical software package SAS (version 8.0 or greater) will be used
- > for analysis."
- >
- > Page 11 Move the sample size justification to the page 5 and replace the statement there with:
- >
- > The test of equivalency of survival rates assumes 100% survival in both isoforms and a 20%
- > maximum difference resulting in a sample size of 15 of each sex per isoform. The test of equivalency of immune response assumes a log10 standard deviation of 0.50 logs and a
- > maximum ratio of geometric mean titers of 4-fold (0.60 logs) resulting in a sample
- > size of 12 of each sex per isoform. Therefore, 30 rabbits (15 male and 15 female) must be tested at each isoform and with the combined isoforms as a control. The unprotected
- > controls
- > will be set at a nominal 4 animals (2 of each sex) due to confidence in the 100% lethality of the challenge dose."
- >
- >
- > Reference: Farrington CP and Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Statistics in Medicine, vol 9, 147-1454, 1990.
- >
- >
- >

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: anti-rPA serum
Date: Wednesday, September 19, 2001 8:13:16 AM

Hi, (b) (6),

We will have both placebo and vaccine ready for pickup on Monday, September 24. We will make enough placebo to cover future immunizations as well, since there is no worry about antigen change or degradation. You may give my phone number (b) (6) or email out, but due to security precautions, they won't be able to get on post. I will meet them at the Post office on 7th Street. They will come to Frederick via 270N, then continue on 15N. The exit they will take will be the 7th Street exit. They should bear to the right, and turn right onto 7th Street (towards Fort Detrick, not the hospital, which is towards the left.) They will go under the Route 15 overpass and take the first right into the post office parking lot. I will meet them there. If they need a map, I can send them one by FAX.

- Sincerely,

Bruce Ivins

-----Original Message-----

From: (b) (6)
Sent: Tuesday, September 18, 2001 5:08 PM
To: 'Bruce.Ivins@DET.AMEDD.ARMY.MIL'
Subject: anti-rPA serum

Bruce,

It is time again to bother you. Sorry! Next booster of the 500 rPA-animals is scheduled for Tuesday, September 25 (6 micrograms in 0.5 ml doses). Our last meeting was so hectic (by the way, thanks much for all your help) that I forgot to request from you the placebo for the control group. This consists of 30 mice that will be bled in groups of ten each, three weeks after one, two or three doses of placebo (0.5 ml of same vaccine diluent containing same amount of aluminum than rPA-vaccine). Hopefully you can provide us with this latter prep this time (enough for all 30 mice; bleeding of the last group will be out of phase three weeks, but I don't think it's a big deal). One last thing. We are paying the contractor for picking up material; last time I delivered the vaccine myself in person, but in this instance I would prefer that Biocon picks it up directly from you. Is that possible? Please let me know, and if you don't mind I will give them your phone to make arrangements for pick up directly. Thanks much and best regards.

(b) (6)



From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: UBMTA to sign
Date: Wednesday, September 19, 2001 8:40:58 AM

Thanks, (b) (6)
- Bruce

>-----Original Message-----

>From: (b) (6)
>Sent: Wednesday, September 19, 2001 8:40 AM
>To: Ivins, Bruce E Dr USAMRIID
>Subject: UBMTA to sign

>

>Hi Bruce,

>I received the signed UBMTAs from (b) (6). Whenever you get a chance can you please come by and sign & date the agreements. I'll be at a meeting this morning. I'll leave them on my chair. Before even receiving them, I started the routing initials (as you know it takes awhile). Once I get (b) (6) okay, we're good to go. (6)

>

(b) (6)

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: SPores
Date: Thursday, September 20, 2001 9:41:06 AM

(b)

If you think it would be helpful, I would be willing to come up to Battelle to offer advice or suggestions with respect to spore production, purification, storage, etc. When we first started working on it years ago, it took us quite awhile to learn the "art" and techniques involved in getting spores which were, stable, pure, unclumped, etc. Honestly, in 1 day to no more than a week, I might be able to save more than several weeks to several months worth of work on your part. Although spore production and purification sounds, on the surface, very easy and cookbook, there are some little nuances in the methodology which are important, yet hard to write down.

Seriously, (b) please let me know if you'd like me to come, and if so, when. At a time like this, I think we all need to come together in single directed purpose, and if that includes my coming there for one or a few days, I'd be happy to do it.

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: Research Progress report
Date: Thursday, September 20, 2001 9:52:09 AM
Attachments: [REDACTED]

I have enclosed my research progress report, with changes made as required by the Anthrax Steering Committee.

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: FW: SPores
Date: Thursday, September 20, 2001 10:12:05 AM

(b) (6)

I just sent this to (b) (6) and I thought I'd send it to you. If you think it might be helpful for me to make a trip to Battelle for advice, suggestions, etc. on spore purification, harvest, storage, etc., I'd be happy to come. It's as much an art as a science, and I could be there for whatever parts of the process you'd like. The purification on gradients is especially tricky.

- Bruce

>-----Original Message-----

>From: Ivins, Bruce E Dr USAMRIID
>Sent: Thursday, September 20, 2001 9:41 AM
>To: (b) (6)
>Subject: SPores

>

(b) (6),

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>

> Seriously, (b) (6) please let me know if you'd like me to come, and if so, when. At a time like this, I think we all need to come together in single directed purpose, and if that includes my coming there for one or a few days, I'd be happy to do it.

>

>- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Contract Mod
Date: Thursday, September 20, 2001 10:20:26 AM

Thanks, (b) (6)

I also think that a parenteral challenge will be the best in this situation, having been involved in both parenteral and aerosol challenges.

- Bruce

-----Original Message-----

From: (b) (6)
Sent: Thursday, September 20, 2001 10:18 AM
To: (b) (6)
Cc: Ivins, Bruce E Dr USAMRIID; (b) (6)
Subject: RE: Contract Mod

(b) (6)

We are completing the protocols and I plan to send them to you by tomorrow. They will not be in SOP form but instead in rougher form, as you call "study specific methods." I want to preview for you the following changes not mentioned in the SOW:

1. Two methods for desorption of rPA will be used as follows. Sodium carbonate for protein to be assayed by SDS-PAGE, Western, and RP-HPLC. Sodium Phosphate for assay by native PAGE (Phast Gel). These procedures are identical except for the solution as indicated. The rationale is that while sodium carbonate will desorb much of the rPA its biophysical form is altered as seen by native PAGE but not by the other assays. Sodium phosphate will elute rPA that is identical to unadsorbed rPA, but the yield is only about 50%. So, we will look at the bulk of the adjuvanted protein with assays that are not sensitive to slight changes in charge, and look at a smaller portion of the adjuvanted protein using an assay that will distinguish between charge forms.
2. The tech base committee recommended injection rather than aerosol for exposure due to much greater precision in delivery of spores and subsequent confidence in statistical analysis of results. However, I am still awaiting a "go" from our prime systems contractor. I know this would change the costing and planning, so I will get back to you as soon as I get a final word from those in higher authority.

(b) (6)

-----Original Message-----

From: (b) (6)
Sent: Monday, September 17, 2001 1:20 PM
To: (b) (6)
Cc: (b) (6)
Subject: Contract Mod

Hi (b) (6) On Friday we received the contract mod from the USAMRMC Contracting Officer to start your stability project. We have started to write the animal use protocol and we ordered the PhastSystem today. Any idea when you can provide us with all the SOPs and Methods that we need to have in place (in our format) before we can start the work? We also need to schedule the first teleconference - perhaps a better use of everybody's time if we schedule it after you have sent us the SOPs and we have had a couple of days to review them so that we can ask relevant questions.

Thanks

(b) (6)

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Cc: (b) (6)
Subject: rPA
Date: Thursday, September 20, 2001 2:41:41 PM

(b) (6),

Thank you for the 30 vials of rPA, lot 100506, 1.18 mg/ml, that you gave me this morning for tech-base studies in support of the development of a new human anthrax vaccine. It will be put to use immediately.

- Bruce Ivins

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Cc: (b) (6)
Subject: B98-03 rabbit bacteremia data
Date: Tuesday, September 25, 2001 9:13:34 AM
Attachments: [REDACTED]

Here are the B98-03 rabbit bacteremia data in an EXCEL file.
- Bruce

From: ivins_bruce@usamriid.hhs.gov
To: (b) (6)
Subject: Money for long-term rabbit study
Date: Tuesday, September 25, 2001 11:22:04 AM

(b) This is the money I come up with for the long-term rabbit study. What is needed for the long-term monkey study and the strain study will be forthcoming. The figures are for supplies, animals, etc. necessary to do the study. Personnel money (salaries, travel, etc.) is not included.

Costs:

192 rabbits.....	\$21,120
Aerosolization costs.....	\$1,920
Supplies costs (Tyveks, gloves, booties, head covers, syringes, needles, tubes).....	\$10,000
ELISA and TNA costs.....	\$3,400
Husbandry costs (daily rabbit care).....	\$296,640
Total.....	\$330,080

.....
(b) as just given me her costs for the diverse strain study:

Costs:

200 rabbits.....	\$22,000
Animal husbandry (100 days).....	\$60,000
Aerosolization costs.....	\$2,000
Supplies.....	\$10,000
ELISA and TNA assays.....	\$1,000
Total.....	\$95,000

I'll get the monkey study info to you as soon as possible.
- Bruce

From: ivins_bruce@usamriid.hhs.gov
To: [REDACTED]
Subject: Money for long-term rabbit study
Date: Tuesday, September 25, 2001 11:22:04 AM

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- Bruce

From: [Ivins, Bruce E. Dr. USAMRIID](mailto:Ivins_Bruce.E.Dr.USAMRIID)
To: [Ivins, Bruce E. Dr. USAMRIID](mailto:Ivins_Bruce.E.Dr.USAMRIID); (b) (6)
Subject: RE: Money for long-term rabbit study
Date: Tuesday, September 25, 2001 4:07:00 PM

Monkey long-term efficacy study money requirements. Please keep in mind that the costs are for 3 years. We plan to use 10 monkeys + 2 controls per time point (3 months, 6 months, 12 months, 24 months, 25 months (if 24 month survival is less than 80%), 36 months, and 37 months (if 36 month survival is less than 80%).

Cost:

72 monkeys... (\$3,000 per monkey).....	\$216,000
Aerosolization costs.....	\$1,000
Supplies.....	\$10,000
ELISA and TNA costs.....	\$2,000
Husband costs (\$6.00 per day per animal).....	\$822,240
Total.....	\$1,051,240

If the costs are deemed too high, then we can lower the number of timepoints, thus lowering the total number of monkeys.

- Bruce

>-----Original Message-----
>From: Ivins, Bruce E Dr USAMRIID
>Sent: Tuesday, September 25, 2001 11:22 AM
>To: (b) (6); Ivins, Bruce E Dr USAMRIID; (b) (6)
>Subject: Money for long-term rabbit study

> This is the money I come up with for the long-term rabbit study. What is needed for the long-term monkey study and the strain study will be forthcoming. The figures are for supplies, animals, etc. necessary to do the study. Personnel money (salaries, travel, etc.) is not included.

>

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>- Bruce

From: [Ivins, Bruce E Dr USAMRIID](mailto:Ivins_Bruce.E.Dr.USAMRIID)
To: [Ivins, Bruce E Dr USAMRIID](mailto:Ivins_Bruce.E.Dr.USAMRIID); (b) (6)
Subject: RE: Money for long-term rabbit study
Date: Tuesday, September 25, 2001 4:07:00 PM

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>To: (b) (6); Ivins, Bruce E Dr USAMRIID; (b) (6)
>Subject: Money for long-term rabbit study

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>I'll get the monkey study info to you as soon as possible.

>- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: Strains
Date: Monday, October 01, 2001 2:41:38 PM

Hi, (b) (6)

Thanks for your phone call earlier today. (b) (6) and I both gave some thought as to the B. anthracis strains of interest with respect to sequencing. Am I correct that the Ames strain has been sequenced? It certainly would be a fine candidate for a "type strain." The other strains would be

- 1) Vollum 1B
- 2) The Australia strain that you and (b) (6) sent (b) (6) (It killed all 16 immunized guinea pigs.)
- 3) The Kruger A isolate
- 4) The Kruger B isolate

I think that if you do one of the Kruger isolates, you should do the other. The Vollum 1B strain is of interest because it has been so extensively used, and because it is demonstrably less virulent than the Ames strain in certain animal models. The Australia strain is of interest because of its exceptional virulence in the immunized guinea pig.

Hope this is helpful. If you'd like to talk about these any more, I'd be happy to do so.

Have a great fall!

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: Florida case(?)
Date: Thursday, October 04, 2001 9:57:19 PM

Hi, (b) (6)

I just heard this evening (and read over internet news) that a case of pulmonary anthrax may have been identified in Florida. Is this true, or is this just hysteria? The only Florida strain of *B. anthracis* that I am familiar with is V770, which is the parent of V770-NP1-R, the strain used in production of the human anthrax vaccine. (I believe that V770 was originally isolated from a cow in Florida in the early 1950s.) The article said that this person was an "Outdoorsman," and had drunk water from a creek in North Carolina. If he really does have anthrax, could he have gotten it this way, or did he get it by tromping around some dusty field area. (Has North Carolina been dry this summer?) I know that in the wild in Africa, animals are supposed to be able to get it from water holes by stirring up spores and presumably ingesting and possibly inhaling them as an aerosol. Could this have happened? What if an animal had died upstream and the stream was contaminated? (Drinking from a stream or creek without boiling or purifying the water first is an invitation to intestinal disease or parasites, but have any other human anthrax cases been documented from people drinking contaminated water?)

You called me several times in the recent past (b) (6) with regards to another anthrax issue. If there's anything I can help with here (if you or coworkers are involved) please let me know. I don't know if there's anything I can do, but I'm certainly willing to provide whatever informational assistance I can. (I would have been less surprised if the Florida man had been hunting deer in Texas, where there is identifiable anthrax. I don't recall North Carolina as having ideal soil for preservation of anthrax spores or for anthrax cycling of spore-vegetative cell-spore-vegetative cell etc., but I suppose there could be areas of higher soil calcium and alkalinity.)

Anyway, please don't hesitate to give me a call if there's anything I can do. We are currently testing the virulence (in immunized and unimmunized guinea pigs) of *B. anthracis* strains from all over the world, including China, and we've come up with some very interesting differences in virulence among the strains.

Take care of yourself, (b) (6)

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 10:52:45 AM

The data we are getting from our with formaldehyde/without formaldehyde experiment in rabbits is giving us VERY strong evidence that we should incorporate a stabilizer in with rPA and Alhydrogel. (b) (6) weren't some FDA-acceptable stabilizers going to be identified? If there some out there, maybe we should start thinking about them now.

Basically what we have as far as the experiment:

1) Five years ago rPA/Alhydrogel/PBS vaccine was made with or without 0.02% formaldehyde (the level that's in AVA) and stored at 4C. With these vaccines we immunized groups of rabbits as follows (0.5 ml per intramuscular dose):

Group A - 24 rabbits (12 males, 12 females) get PA (50 ug)/Alhydrogel (0.5 mg)/PBS/0.02% formaldehyde at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

Group B - 24 rabbits (12 males, 12 females) get PA (50 ug)/Alhydrogel (0.5 mg)/PBS/No formaldehyde at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

Group C - 4 rabbits (2 males, 2 females) get PBS/Alhydrogel (0.5 mg) at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

2) Results so far, 3 days after challenge:

Group	Survived/Total
A - Vaccine plus formaldehyde	24/24 (no deaths)
B - Vaccine minus formaldehyde	16/24 (8 deaths)
C - Controls	0/4 (4 deaths)

Note: We originally studied the effect of formaldehyde on rPA vaccine potency/stability in guinea pigs. The cumulative data indicated that stability/potency was enhanced by the presence of formaldehyde.

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:04:56 AM

No, just survival data.
- Bruce

-----Original Message-----

From: (b) (6)
Sent: Friday, October 05, 2001 10:56 AM
To: Ivins, Bruce E Dr USAMRIID; (b) (6)

(b) (6)
Subject: RE: Stabilizer in a new rPA vaccine

Bruce,

Do you have any data yet on the protein composition (i.e. is it degraded) in the with or without formaldehyde?

(b) (6)

(b) (6)

-----Original Message-----

From: Ivins, Bruce E Dr USAMRIID [<mailto:Bruce.Ivins@DET.AMEDD.ARMY.MIL>]
Sent: Friday, October 05, 2001 10:53 AM
To: (b) (6)

(b) (6)
Subject: Stabilizer in a new rPA vaccine

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Note: We originally studied the effect of formaldehyde on rPA vaccine potency/stability in guinea pigs. The cumulative data indicated that stability/potency was enhanced by the presence of formaldehyde.

- Bruce

From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:04:56 AM

No, just survival data.
- Bruce

-----Original Message-----

From: (b) (6)
Sent: Friday, October 05, 2001 10:56 AM
To: Ivins, Bruce E Dr USAMRIID; (b) (6)

(b)
Subject: RE: Stabilizer in a new rPA vaccine

Bruce,

Do you have any data yet on the protein composition (i.e. is it degraded) in the with or without formaldehyde?

(b)
(6)

(b) (6)

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To: [Ivins, Bruce E Dr USAMRIID](#); (b) (6)
Subject: FW: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:07:04 AM

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From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:10:17 AM

I don't have an earlier time point in rabbits, just guinea pigs.

- Bruce

>-----Original Message-----

>From: (b) (6)
>Sent: Friday, October 05, 2001 11:10 AM
>To: Ivins, Bruce E Dr USAMRIID
>Subject: RE: Stabilizer in a new rPA vaccine

>

>Do you have an earlier time point? Is this the same batch of protein that (b) (6) showed to be more degraded with formaldehyde than without?

>

>-----Original Message-----

> From: Ivins, Bruce E Dr USAMRIID
> Sent: Friday, October 05, 2001 11:07 AM
> To: Ivins, Bruce E Dr USAMRIID; (b) (6)

[Redacted]

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From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:21:42 AM

That is (b) (6) domain.
- Bruce

-----Original Message-----

From: (b) (6)
Sent: Friday, October 05, 2001 11:02 AM
To: Ivins, Bruce E Dr USAMRIID; (b) (6)

Subject: RE: Stabilizer in a new rPA vaccine

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From: [Ivins, Bruce E Dr USAMRIID](#)
To: (b) (6)
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Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 11:31:44 AM

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To: (b) (6)
Subject: RE: Stabilizer in a new rPA vaccine
Date: Friday, October 05, 2001 2:29:40 PM

All - After the final data are in, shall we plan a meeting to discuss where we (tech base/advanced development) go from here with respect to incorporating a stabilizer in the vaccine? Possible questions to address:

- 1) What if any studies are necessary to follow up these data?
- 2) What stabilizers are acceptable to the FDA?
- 3) What path should we take as far as incorporating a particular stabilizer into a new anthrax vaccine?
- 4) Others(???)

- Bruce

-----Original Message-----

From: (b) (6)
Sent: Friday, October 05, 2001 12:17 PM
To: (b) (6)

(b) (6)
Subject: RE: Stabilizer in a new rPA vaccine

In my opinion, this shows that half of the rPA had spontaneously desorbed from the Alhydrogel after 5 years, and remained undegraded in the presence of formaldehyde (lane 9), or completely degraded in the absence of formaldehyde (lane 8). Protein that remained on the Alhydrogel multimerized in the presence of formaldehyde (lane 4) or remained largely intact in the absence of formaldehyde, while at the same time having a larger number of breakdown products of small concentration (lane 5). Both of the latter samples contain the familiar charge isoforms of full-length rPA protein.

-----Original Message-----

From: (b) (6)
Sent: Friday, October 05, 2001 11:51 AM
To: Ivins, Bruce E Dr USAMRIID; (b) (6)

Subject: RE: Stabilizer in a new rPA vaccine

Here is the gel on rPA desorbed from 5 year old vaccine. (b) (6)

-----Original Message-----

From: Ivins, Bruce E Dr USAMRIID
Sent: Friday, October 05, 2001 11:22 AM
To: (b) (6)

(b) (6)

Subject: RE: Stabilizer in a new rPA vaccine

That is (b) (6) domain.

- Bruce

-----Original Message-----

From: (b) (6)

Sent: Friday, October 05, 2001 11:02 AM

To: Ivins, Bruce E Dr USAMRIID; (b) (6)

Subject: RE: Stabilizer in a new rPA vaccine

You will still need the protein data to confirm that it is a stability problem and not an adjuvating affect.

(yeah, I know, I always have to play devil's advocate).

(b)

(6)

-----Original Message-----

From: Ivins, Bruce E Dr USAMRIID [<mailto:Bruce.Ivins@DET.AMEDD.ARMY.MIL>]

Sent: Friday, October 05, 2001 11:07 AM

To: Ivins, Bruce E Dr USAMRIID; (b) (6)

Subject: FW: Stabilizer in a new rPA vaccine

It's up to everyone else. It does strongly appear as though we will need a stabilizer, however.

- Bruce

> -----Original Message-----

> From: (b) (6)

> Sent: Friday, October 05, 2001 11:05 AM

> To: Ivins, Bruce E Dr USAMRIID

> Subject: RE: Stabilizer in a new rPA vaccine

>
> Bruce,
> This could figure into the upcoming stability/efficacy study, for which I specifically asked about an concurrent set with an excipient but DVC considered this too early. Should we meet?

(b) (6)

>
> -----Original Message-----
> From: Ivins, Bruce E Dr USAMRIID
> Sent: Friday, October 05, 2001 10:53 AM
> To: (b) (6)

> Subject: Stabilizer in a new rPA vaccine

>
> The data we are getting from our with formaldehyde/without formaldehyde experiment in rabbits is giving us VERY strong evidence that we should incorporate a stabilizer in with rPA and Alhydrogel. (b) (6)

..weren't some FDA-acceptable stabilizers going to be identified? If there some out there, maybe we should start thinking about them now.

>
> Basically what we have as far as the experiment:

>
> 1) Five years ago rPA/Alhydrogel/PBS vaccine was made with or without 0.02% formaldehyde (the level that's in AVA) and stored at 4C. With these vaccines we immunized groups of rabbits as follows (0.5 ml per intramuscular dose):

> Group A - 24 rabbits (12 males, 12 females) get PA (50 ug)/Alhydrogel (0.5 mg)/PBS/0.02% formaldehyde at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

> Group B - 24 rabbits (12 males, 12 females) get PA (50 ug)/Alhydrogel (0.5 mg)/PBS/No formaldehyde at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

> Group C - 4 rabbits (2 males, 2 females) get PBS/Alhydrogel (0.5 mg) at 0 weeks. Challenge (subcutaneous) at 6 weeks with about 100 LD50 Ames spores.

>
> 2) Results so far, 3 days after challenge:

>
> Group Survived/Total
>
> A - Vaccine plus formaldehyde 24/24 (no deaths)
>
> B - Vaccine minus formaldehyde 16/24 (8 deaths)
>
> C - Controls 0/4 (4 deaths)

>
>
> Note: We originally studied the effect of formaldehyde on rPA vaccine potency/stability in guinea pigs. The cumulative data indicated that stability/potency was enhanced by the presence of formaldehyde.

>
> - Bruce