B01-11

Formaldehyde Studies

~~Isoform Studies~~ No B2

Other PA vaccine Studies

Department Bacteriology #4241

Subject ~~AVA~~ ~~PPA~~ PA experiments

Name Bruce Ivins

Address Issued 2/16/00

National® Brand

Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets 43-648

0 73333 43648 8

AVERY DENNISON

Office Products
Chicopee, MA 01022

070628002-HC
Q2135
RWW

Recombinant PA experiments

Book 4241 - B01-11; Formaldehyde studies; Other PA vaccine studies

Page

7 - Effect of PBS on the pH of ammonium acetate solution
9 - rPA lot 100506, receipt of 30 vials from John Hewetson
11 - B00-01 Statement of work
13 - B00-01 quote from Covance
15 - Explanation of "Old Formaldehyde" experiment
17 - 29 - Challenge data for "Old Formaldehyde" experiment
31 - Addendum request for rabbits in "New Formnaldehyde" experiment
33 - Explanation of "New Formaldehyde" experiment
35 - Preparation of rPA + formaldehyde vaccine
35 - Final survival data after challenge
37 - 43 - Individual animal data

q2135.2

q2135.1

Thus →

It appears as though the addition of PBS has little effect on the pH of the ammonium acetate

— Bruce Irvin 4/2/01

Ammonium acetate + PBS – pH effects

rPA is currently purified into pH 8.9, 50mM ammonium acetate. Then it is diluted in Dulbeccos PBS (no calcium or magnesium) to the desired rPA concentration.

Question: what's the effect on pH of mixing of PBS with 50mM ammonium acetate, pH 8.9 - and so we did the following experiment:

1. We made 50mM ammonium acetate, pH 8.9
2. We mixed the ammoniumacetate solution with PBS to a total volume of 100ml and noted pH.

Amount of PBS	Amount of ammoniumacetate	pH
100ml	0ml	7.36
90ml	10ml	8.65
80ml	20ml	8.90
70ml	30ml	8.97
60ml	40ml	9.00
50ml	50ml	9.02
40ml	60ml	9.02
30ml	70ml	9.02
20ml	80ml	9.02
10ml	90ml	9.00
0ml	100ml	9.00

[illegible] 4/12/01

rPA - lot 100506. 30 vials
obtained from (b)(6) [redacted] on 20 Sep 2001
Currently on Hand - 30 vials - 20 Sep 01 BR
B00-01

9

COVANCE RESEARCH PRODUCTS INC.

REQUEST FORM

So that we can meet your requirements, please provide as much information as possible.
If you have any questions, feel free to call us, and we will be happy to assist you.

Investigator: Bruce Irvins (b)(6) [redacted] QUOTE #
Salutation: Dr. ☒ Mr. ☐ Mrs. ☐ Ms. ☐ Miss ☐ E-Mail Address:
Phone #: (b)(6) [redacted] Fax #: (b)(6) [redacted]
P.O. #: P.O. Limit: Exp. Date:
Purchasing Agent: Purchasing Phone #: /

Project Information	
Name of Immunogen: recombinant PA (Alhydrogel)	
Format (e.g. liquid, powder, gel, etc.)	
Storage Conditions: 4°-6°C	
# of Vials:	
Volume Per Vial:	
Concentration:	
Human/Animal Health Hazard: ☐ Yes ☒ No	
Precautions: None	
Animal Species Requested: Rabbits	
# of Animals Needed/Immunogen: 52	
Optional: # of Animals to be pre-screened ☐ None required	
Use CRP Immunization /Bleed Protocol: ☐ Yes ☐ No or ☒ Special instructions* attached	
*Please note that prior to starting a project, each protocol must be approved by the institution's IACUC and Covance as it relates to the animal welfare at Covance Research Products, Inc. Animal Protocol B01-11	

Mailing Address	
Contact	
Company/Institution	
Dept./Bldg./Room #	
Address 1	
Address 2	
City, State, Zip	
Shipping Address (if different)	
Contact	
Company/Institution	
Dept./Bldg./Room #	
Address 1	
Address 2	
City, State, Zip	
Billing Address (if different)	
Contact	
Company/Institution	
Dept./Bldg./Room #	
Address 1	
Address 2	
City, State, Zip	

Immunochemistry Services:
A. ELISA: ☐ Yes ☒ No Screening Antigen:
B. Purification: ☐ Yes ☒ No Type: Protein A ☐ Protein G ☐ Affinity ☐ Other
C. Conjugation: ☐ Yes ☒ No Type: KLH ☐ BSA ☐ Thyroglobulin ☐ Other
Note: Conjugation is recommended for both immunogen and screening antigen peptides < 25 amino acids in length.
D. Peptide Synthesis: ☐ Yes ☒ No Sequences:
Special Instructions:

Signature: Bruce Irvins Date: 6 Aug 01

EAST COAST
Covance Research Products Inc.
Immunology Services
PO Box 7200
465 Swampbridge Road
Denver, PA 17517
800/345-4114 • 717/336-4921 • Fax: 717/336-3481

WEST COAST
Covance Research Products Inc
Immunology Services
1223 South 47th Street
Richmond, CA 94804
800/922-2226 • 510/412-8930 • Fax: 510/412-8940

92135, 10

Bruce Irvins - 4 Oct 01

92135, 9

B00-01

STATEMENT OF WORK

EFFECT OF FORMALDEHYDE ON THE IMMUNOGENICITY OF AN ANTHRAX rPA VACCINE IN RABBITS

Conditions to be tested:

1. The rPA vaccines (0.5 ml per i.m. dose) will be formulated with 50 μg rPA, aluminum hydroxide (0.5 mg aluminum) and either 0.02% or 0% formaldehyde. Controls will be formulated without rPA or formaldehyde.

2. Rabbits (52 total; 26 male and 26 female) have been purchased already from Covance Research Products, order number 1137-0002, by USAMRIID.

3. Rabbits will be divided into 3 groups: A, B and C. They will be numbered with a unique identifier to included group designation, either A, B or C, as a prefix, and the sex of the animal, either M or F, as a suffix. The 24 (12 male and 12 female) rabbits in group A will receive vaccine containing formaldehyde. The 24 (12 male and 12 female) rabbits in group B will receive vaccine without formaldehyde. The 4 (2 male and 2 female) rabbits in group C are controls, and will receive PBS + Alhydrogel without rPA or formaldehyde.

4. All rabbits will be injected i.m. with 0.5 ml of the appropriate vaccine or control material on Tuesday, 21 AUG 01 (week 0).

5. Rabbits will be bled for 5 ml of blood at weeks 2 and 4.

6. Rabbits will be shipped to USAMRIID on week 5 (24 ~~AUG~~ Sep 01).

TABLE

Group	# Rabbits	Injection	Numbers
A	24	rPA/Alhydrogel/formaldehyde	12 M, 12 F
B	24	rPA/Alhydrogel/No formaldehyde	12 M, 12 F
C	4	PBS/Alhydrogel	2M, 2F

Q2135,20

Bruce Lims - 4 Oct 01

Q2135,19

Bu

COVANCE
THE DEVELOPMENT SERVICES COMPANY

CONFIDENTIAL

Covance Research Products
P.O. Box 7200
Denver, PA 17517
Tel: 800/345-4114
717/336-4921
Fax: 717/336-3481

Rabbit Protocol - Quote # 01-0833 **Investigator: Dr. Ivins**

Protocol

Day:	Procedure:	Cost:
0	NZW Female Rabbit	$59.35
	Prebleed (Avg. 5 ml serum)	11.35
	1° IM: 0.5 ml of vaccine	15.35
14	Test Bleed (Avg. 5 ml serum)	11.35
28	Test Bleed (Avg. 5 ml serum)	11.35
	Maintenance: $1.35/day x 30 days	40.50
	Cost per rabbit	$149.25
	Cost for 52 rabbits	$7761.00
	Ship 52 rabbits via CRP truck	$265.18
	Box Charge $10.50 ea x 52	$546.00
		$811.18
	Total	$8572.18

Changes can be made to enhance the animal's response, which is solely dependent upon the antigenicity of the immunogen. Please feel free to make any changes to the above protocol by adding, deleting, or changing any of the procedures, as well as the timing between procedures. Any changes made however, will have a direct effect, either increase or decrease, on the total cost of the program.

Effective 01/12/01

Confidentiality Notice: This facsimile transmission may contain confidential or legally privileged information which is intended only for the use of the individual or entity named on this transmittal sheet. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution, or reliance upon the contents of this facsimile is strictly prohibited. If you have received this facsimile transmission in error, please notify us immediately by telephone so that we can arrange for the return of the transmitted materials to us at no cost to you.

92135.30

Bruce Ivins - 4 Oct 01

92135.28

Sub Cu challenge
2 OCT-01
Spores on 1 OCT
Plate count is 3 OCT

B01-11

This is a study to determine whether there is any potency difference in anthrax vaccine ± formaldehyde stored since 1996 and used in B96-08.

Groups of rabbits (2.5-3.5kg, NZW, Covance) will be immunized at 0 weeks & bled at 0, 2 & 4 weeks, then challenged s.c. at 4 weeks c̄ about 100 LD_{50} Ames spores.

Actual challenge dose = 1.94×10^5 spores = 124 LD_{50}

Group A - 12 males, 12 females get, at 0 wk, 0.5 ml containing rPA (50 ug), Alhydrogel (0.5 mg Aluminum), 0.02% formaldehyde, (in PBS)

Group B - 12 males, 12 females get, at 0 wk, 0.5 ml containing rPA (50 ug), Alhydrogel (0.5 mg Aluminum), 0.02% formaldehyde (in PBS).

Group C - PBS + Alhydrogel Control - 4 rabbits (2 each gender) get injected at 0 wk

Numbers next page

Bruce Davis - 5 Oct 01.

A = + formaldehyde

B = − formaldehyde

C = control

Code	Group	Sex	Day of Death
AF273MA	A	M	?
AF274MA	A	M	
AF275MA	A	M	
AF276MA	A	M	
AF277MA	A	M	
AF278MA	A	M	
AF279MA	A	M	
AF280MA	A	M	
AF281MA	A	M	
AF282MA	A	M	
AF283MA	A	M	
AF284MA	A	M	
AF285FC	A	F	
AF286FC	A	F	
AF287FC	A	F	
AF288FC	A	F	
AF289FC	A	F	
AF290FC	A	F	
AF291FC	A	F	
AF292FC	A	F	

Brandina 8 Feb 02

Code	Group	Sex	Day of Death
AFT293FE	A	F	?
AFT294FE	A	F	
AFT295FE	A	F	
AFT296FE	A	F	
BFT297MA	B	M	
BFT298MA	B	M	
BFT299MA	B	M	
BFT300MA	B	M	
BFT301MA	B	M	
BFT302MA	B	M	
BFT303MA	B	M	
BFT304MA	B	M	
BFT305MA	B	M	
BFT306MA	B	M	
BFT307MA	B	M	
BFT308MA	B	M	
BFT309FE	B	~~M~~ F (β2de-5oct01)	
BFT310FE	B	~~M~~ F (β2de-5oct01)	
BFT311FE	B	F	

Bruce Irvis - 8 Feb 02

Code	Group	Sex	Day of Death
BFT312FE	B	F	?
BFT313FE	B	F	
BFT314FE	B	F	
BFT315FE	B	F	
BFT316FE	B	F	
BFT317FE	B	F	
BFT318FE	B	F	
BFT319FE	B	F	
BFT320FE	B	F	
CFT321MA	C	M	
CFT322MA	C	M	
CFT323FE	C	F	
CFT324FE	C	F	✓

Bruce Irvin - 8 Feb 02

Effect of Formaldehyde on the Potency of RPA Vaccine in Rabbits

New Zealand White (NZW) rabbits were inoculated at 0 weeks with rPA/Alhydrogel in PBS **(plus formaldehyde)** or rPA /Alhydrogel in PBS **(minus formaldehyde).** (The vaccines were prepared 5 years ago and stored at 4°C.) At 2 weeks and 4 weeks the rabbits were bled for ELISA titers. At 6 weeks the animals were challenged subcutaneously with approximately 125 LD_{50} of virulent anthrax spores. Deaths were recorded for 3 weeks.

Vaccines:

a) rPA (50 µg) + Alhydrogel (0.5 mg aluminum per 0.5 ml dose) + PBS plus 0.02% formaldehyde
b) rPA (50 µg) + Alhydrogel (0.5 mg aluminum per 0.5 ml dose) + PBS minus 0.02% formaldehyde
c) PBS + Alhydrogel (0.5 mg aluminum per 0.5 ml dose)

Rabbit Groups:

a) 24 rabbits (12 males, 12 females) get rPA vaccine plus formaldehyde at 0 weeks. Subcutaneous challenge at 6 weeks with 125 LD_{50} virulent spores
b) 24 rabbits (12 males, 12 females) get rPA vaccine minus formaldehyde at 0 weeks. Subcutaneous challenge at 6 weeks with 125 LD_{50} virulent spores
c) 4 control rabbits (2 males, 2 females) get Alhydrogel + PBS

Results:

Group	Survived/Total	(%)	2-wk titer	4-wk titer
Vaccine + form.	16/24	(67)	89	73
Vaccine - form.	11/24	(46)	74	15
Controls	0/~~24~~ 4 B2-de - 11 Feb 02	(0)	---	---

(Micrograms anti-PA per ml of serum)

SDS PAGE Analysis of rPA desorbed from the vaccines revealed that there was little degradation of the PA83 material from the preparations not containing formaldehyde. In the vaccine preparations containing formaldehyde, there appeared to be some aggregation. Formaldehyde does not appear to be needed to keep rPA from breaking down on the Alhydrogel. It may act as an adjuvant by causing local inflammation. This experiment will be repeated with freshly prepared vaccines.

Bruce Ivins - 8 Feb 02

QUANTITATIVE ANTI-PA IgG ELISA- RABBIT PROTOCOL B01-11

		ANTI-PA TITER	
	PRE-BLEED	WEEK 2	WEEK 4
RABBIT #	21-Aug-01	4-Sep-01	18-Sep-01
A-FT 273 M	0.1407	28.77	80.24
A-FT 274 M		39.91	43.44
A-FT 275 M	0.5576	34.44	43.22
A-FT 276 M		71.08	73.58
A-FT 277 M		82.19	123.24
A-FT 278 M	BLQ	34.65	37.27
A-FT 279 M		29.39	59.10
A-FT 280 M		31.33	45.56
A-FT 281 M		145.83	40.28
A-FT 282 M		136.83	43.30
A-FT 283 M		43.68	18.37
A-FT 284 M		295.88	54.41
A-FT 285 F		146.06	31.92
A-FT 286 F	0.1387	55.56	10.84
A-FT 287 F		59.08	37.58
A-FT 288 F		39.58	23.42
A-FT 289 F	0.1227	182.37	58.40
A-FT 290 F		225.03	87.73
A-FT 291 F		145.73	33.63
A-FT 292 F		472.33	93.27
A-FT 293 F		63.58	36.11
A-FT 294 F		304.49	116.07
A-FT 295 F		155.89	39.73
A-FT 296 F		212.01	68.92
AVERAGE		126.49	54.15
GEOMEAN		89.25	46.99

		ANTI-PA TITER	
	PRE-BLEED	WEEK 2	WEEK 4
	21-Aug-01	4-Sep-01	18-Sep-01
B-FT 297 M		163.51	24.20
B-FT 298 M	0.08592	231.00	29.17
B-FT 299 M		174.49	34.36
B-FT 300 M		67.59	8.05
B-FT 301 M		60.03	8.27
B-FT 302 M		128.99	11.28
B-FT 303 M		48.58	8.61
B-FT 304 M	BLQ	183.33	25.60
B-FT 305 M		424.56	53.24
B-FT 306 M		186.78	25.40
B-FT 307 M		78.76	10.32
B-FT 308 M		176.84	23.87
B-FT 309 F	BLQ	175.73	22.53
B-FT 310 F		169.52	19.83
B-FT 311 F		55.91	6.74
B-FT 312 F		261.80	31.21
B-FT 313 F	0.06935	22.91	15.65
B-FT 314 F		45.73	23.86
B-FT 315 F		32.18	12.52
B-FT 316 F		49.75	30.17
B-FT 317 F		17.60	11.03
B-FT 318 F		23.52	8.91
B-FT 319 F	BLQ	9.59	6.56
B-FT 320 F		7.01	6.66
AVERAGE		116.49	19.09
GEOMEAN		73.84	15.97

C-FT 321 M	0.07903	0.09653
C-FT 322 M		BLQ
C-FT 323 F	BLQ	BLQ
C-FT 324 F		BLQ

BLQ = below limits of quantitation

Bruce Lewis - 8 Feb 02

QUANTITATIVE ANTI-PA IgG ELISA- RABBIT PROTOCOL B01-11

RABBIT #	PRE-BLEED 21-Aug-01	ANTI-PA TITER WEEK 2 4-Sep-01	WEEK 4 18-Sep-01
A-FT 273 M	0.1407	28.77	80.24
A-FT 274 M		39.91	43.44
A-FT 275 M	0.5576	34.44	43.22
A-FT 276 M		71.08	73.58
A-FT 277 M		82.19	123.24
A-FT 278 M	BLQ	34.65	37.27
A-FT 279 M		29.39	59.10
A-FT 280 M		31.33	45.56
A-FT 281 M		145.83	40.28
A-FT 282 M		136.83	43.30
A-FT 283 M		43.68	18.37
A-FT 284 M		295.88	54.41
A-FT 285 F		146.06	31.92
A-FT 286 F	0.1387	55.56	10.84
A-FT 287 F		59.08	37.58
A-FT 288 F		39.58	23.42
A-FT 289 F	0.1227	182.37	58.40
A-FT 290 F		225.03	87.73
A-FT 291 F		145.73	33.63
A-FT 292 F		472.33	93.27
A-FT 293 F		63.58	36.11
A-FT 294 F		304.49	116.07
A-FT 295 F		155.89	39.73
A-FT 296 F		212.01	68.92
AVERAGE		126.49	54.15
GEOMEAN		89.25	46.99

C-FT 321 M	0.07903	0.09653	
C-FT 322 M		BLQ	
C-FT 323 F	BLQ	BLQ	
C-FT 324 F		BLQ	

	PRE-BLEED 21-Aug-01	ANTI-PA TITER WEEK 2 4-Sep-01	WEEK 4 18-Sep-01
B-FT 297 M		163.51	24.20
B-FT 298 M	0.08592	231.00	29.17
B-FT 299 M		174.49	34.36
B-FT 300 M		67.59	8.05
B-FT 301 M		60.03	8.27
B-FT 302 M		128.99	11.26
B-FT 303 M		48.58	8.61
B-FT 304 M	BLQ	183.33	25.60
B-FT 305 M		424.56	53.24
B-FT 306 M		186.78	25.40
B-FT 307 M		78.76	10.32
B-FT 308 M		176.84	23.87
B-FT 309 F	BLQ	175.73	22.53
B-FT 310 F		169.52	19.83
B-FT 311 F		55.91	6.74
B-FT 312 F		261.80	31.21
B-FT 313 F	0.06935	22.91	15.65
B-FT 314 F		45.73	23.86
B-FT 315 F		32.18	12.52
B-FT 316 F		49.75	30.17
B-FT 317 F		17.60	11.03
B-FT 318 F		23.52	8.91
B-FT 319 F	BLQ	9.59	6.56
B-FT 320 F		7.01	6.66
AVERAGE		116.49	19.09
GEOMEAN		73.84	15.97

BLQ = below limits of quantitation

Bruce Dunn - 8 Feb 02

SDS PAGE:

Lane 1: Bio-Rad LMW

2: rPA (MARP)

3: rPA desorbed

4: Ivins vaccine 5 yrs old (+formaldehyde)

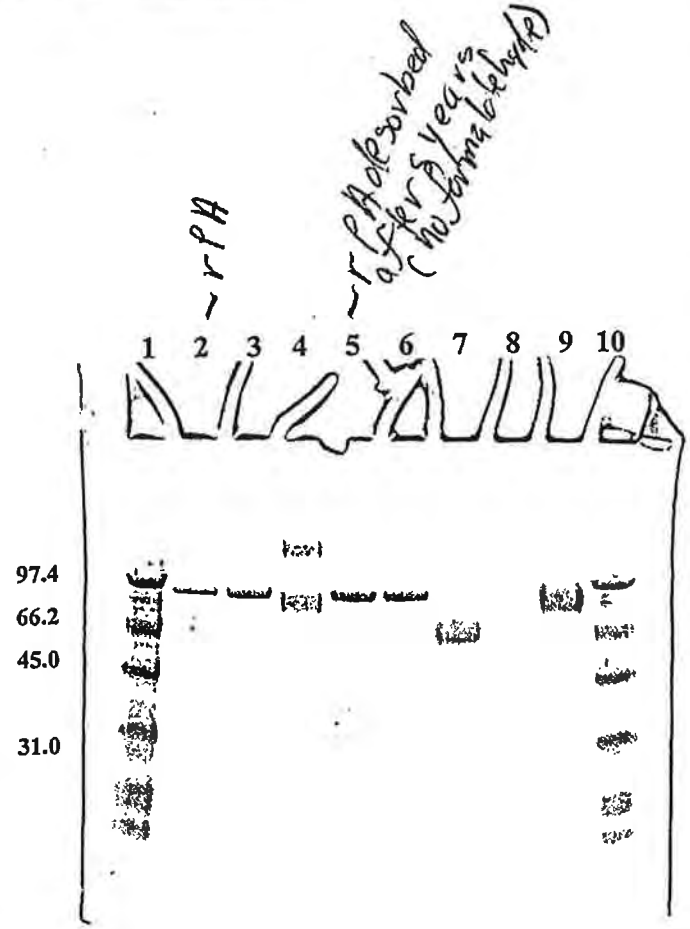
5: Ivins vaccine 5 yrs old (- formaldehyde)

6: rPA (MARP) + carbonate buffer, then Hepes, EDTA

7: BSA + carbonate, then Hepes, EDTA

8: PBS supe from 5 yr old vaccine (no formaldehyde)

9: PBS supe from 5 yr old vaccine (+ formaldehyde)



Bruce Ivins · 8 Feb 00

USAMRIID ANIMAL USE PROTOCOL
PROTOCOL TYPE: ADDENDUM REQUEST

Proposal No....01-28
Protocol No....B01-11
Date Submitted....11 FEB 02

PRINCIPAL INVESTIGATOR: Bruce E. Ivins, Ph.D.

Protocol Title: Effect of formaldehyde on the potency stability of a candidate human anthrax vaccine in rabbits.

1. This protocol was originally approved for 52 rabbits. The ultimate objective of the study was to determine whether formaldehyde should be included in a new recombinant PA (rPA) anthrax vaccine. The specific objective was to determine if there was any potency difference in rPA/Alhydrogel anthrax vaccines (± formaldehyde) prepared in 1996 for protocol B96-08 and stored since then at 4°C. The hypothesis was that we would find that formaldehyde was not required for preservation of potency upon storage of an rPA /Alhydrogel anthrax vaccine.

2. Results of the study: 1) As determined by SDS-PAGE, there was little or no degradation of rPA bound to Alhydrogel in the absence of formaldehyde; 2) There was an increase in survival (not statistically significant) in the group receiving vaccine containing formaldehyde (16/24) compared to the vaccine receiving vaccine without formaldehyde (11/24).

3. The results suggest that formaldehyde may increase survival by acting as an adjuvant (stimulating inflammation at the injection site) rather than by preventing degradation of antigen in the vaccine.

4. We have been informed by the FDA that formaldehyde may be included in a vaccine as a stabilizer but not as an adjuvant. Therefore we are requesting an additional 52 animals to do the proposed additional study:

a) All procedures, including procedures with animals, will be exactly identical to those in the approved B01-11 protocol.

b) The only difference between the proposed addendum and the approved protocol will be that we will perform the experiment with <u>freshly prepared</u> vaccine (± formaldehyde) instead of vaccine that has been stored for 4 years.

c) The results from the proposed study should conclusively demonstrate that formaldehyde does not potentiate the protective immune response to the vaccine by preventing degradation. (Since the vaccine will be used immediately after preparation, any potentiation of the protective immune response will have to come as a result of formaldehyde acting as an adjuvant.) The results of this study should therefore enable us to rationally exclude formaldehyde from a new rPA/Alhydrogel anthrax vaccine for human use.

5. Anticipated numbers in the pain and distress categories are: NO PAIN - 50%; UNALLEVIATED PAIN OR DISTRESS - 50%, based on the results with the initial 52 animals.

Bruce E. Ivins, Ph.D.
Principal Investigator
USAMRIID Bacteriology Division

Bruce Ivins - 11 Feb 02

B01-11, part 2

Experiment: New Formaldehyde Experiment

Animals and Groups:

Group 1) 12 each gender - rPA vaccine with formaldehyde
Group 2) 12 each gender - rPA vaccine without formaldehyde
Group 3) 2 each gender - Alhydrogel/PBS

Immunizations and Vaccine Preparation:

Formaldehyde - Dilute 37% Formalin solution to 2% by adding 1 ml of formalin to 17.5 ml Sterile WFI. This will be diluted 1:100 for group 1 vaccine to make 0.02% formaldehyde.

PA - 1.18 mg/ml.

PBS - no calcium or magnesium

Alhydrogel (2% aluminum oxide)

Group 1) 18 ml total of vaccine. Add 1.53 ml PA, 1.7 ml Alhydrogel, 0.18 ml dilute formaldehyde, and 14.6 ml PBS. Each 0.5-ml i.m. dose will have 50 µg PA, 0.5 mg aluminum and 0.02% formaldehyde.

Group 2) 18 ml total of vaccine. Add 1.53 ml PA, 1.7 ml Alhydrogel, 0.18 ml Sterile WFI, and 14.6 ml PBS. Each 0.5-ml i.m. dose will have 50 µg PA, 0.5 mg aluminum but no formaldehyde.

Group 3) Controls. 5 ml total of PBS + Alhydrogel. Add 0.47 ml of Alhydrogel to 4.53 ml of PBS. This will contain no PA, no formaldehyde, and 0.5 mg of Aluminum per dose.

Dates:

Monday, 16 SEP (afternoon) - make vaccines and control.
Tuesday, 17 SEP (Go to Covance with Vaccines)
Tuesday, 17 SEP (Covance bleeds rabbits - week 0)
Tuesday, 1 OCT (Covance bleeds rabbits - week 2)
Tuesday, 15 OCT (Covance bleeds rabbits - week 4)
Tuesday, 22 OCT (Covance ships rabbits - week 5)
Monday, 28 OCT (We bleed rabbits)
Monday, 28 OCT (We prepare 100 LD50 challenge)
Tuesday, 29 OCT (We challenge 52 rabbits with 100 LD_{50} SC)
Tuesday, 19 NOV (bleed out and euthanize survivors)

On 29 Oct the animals were given 125 LD50 SC of Ames spores

Bruce Ohrris - 17 Dec 00

Here is how we prepare our rPA vaccine with aluminum hydroxide adjuvant:

Materials:

1) Dulbecco's phosphate buffered saline (PBS) without calcium and without magnesium, pH 7.4 - sterile
2) Aluminum hydroxide (Alhydrogel) (2% Aluminum oxide) - sterilized by autoclaving
3) PA in ammonim acetate buffer, pH 8.9

The PA we have been using has a concentration of 1.18 mg/ml. (This can be measured by absorbance at 280 nm, since 1.0 mg/ml of PA gives a reading of 1.0.) We dilute the commercial Alhydrogel stock above 1:10.64 (final dilution in vaccine) to get 0.5 mg of metallic aluminum per 0.5 ml dose of vaccine. (I believe that the maximum allowed is 0.85 mg per dose. The current human anthrax vaccine has about 0.725 mg aluminum per dose. Below is how we would make up 10 0.5-ml doses of vaccine containing 50 micrograms of PA per dose and 0.5 mg of aluminum per dose:

0.424 ml of PA (500 micrograms of the 1.18 mg/ml material)
0.470 ml of Alhydrogel
4.106 ml of PBS

Add the Alhydrogel to the PBS in a sterile vaccine vial and mix by swirling until an even suspension is achieved. Add the PA and swirl immediately to mix. Put on ice and allow to stand from 1 hour to overnight on ice. If desired, a small, sterile stirring bar can be added to the PBS + Alhydrogel. While on a magnetic mixer, add the PA. (We've never had a problem doing it the former way, without a stirring bar.)

Final death count -

Vaccine with formaldehyde - 10 dead /24

Vaccine without formaldehyde ~~14 dead /24~~ 15 dead /24 BZ-de-17Dec02

Controls 4 dead /4

Bruce Ivins 17Dec 02

day 14

LABORATORY WORK COPY

DATE 29 Oct 02

TEST TITLE B01-11

Ivins (Bact. Div.)

ANIMAL Rb's

DOSE 0.2ml SQ

ROUTE SQ

Rx

NOTEBOOK NUMBER 1 PAGE 1 SHEET

	MONTH	Oct		Nov																	
	DATE	30	31	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
	DAYS AFTER INOCULATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GROUP #1 (Marked Red)	5116 M																				
	1268 F																				
	4319 M		X																		
	3854 F																				
	6276 M			X																	
	7597 F																				
	9783 F																				
	5076 F																				
	0881 M																				
	8324 M																				
	1888 M				X																
	8554 M																				
	2830 F																				
	2859 F																				
	2124 F		X																		
	7576 F																				
	1302 F					X															
	0834 F																				
	6531 M				X																
	3038 M																				
	5871 M							X													
	9351 F								X		X										
	4065 M			X																	
	0028 M		X																		

WRAMC FORM 111
15 JAN 1954

(b)(6) [redacted]

Bacteria - 17 Dec 02

15-de-17Dec02

10-group 1 ~~[illegible]~~ 82 after 14 days

LABORATORY WORK COPY		DATE 29 Oct 02
TEST TITLE B01-11	DOSE 0.2 ml	NOTEBOOK
Ivins (Bact. Div.)	ROUTE SQ	NUMBER 1
ANIMAL Rbs	Rx	PAGE 2
		SHEET

	MONTH		OCT		NOV																
	DATE		30	31	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
	DAYS AFTER INOCULATION		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GROUP #2 (Marked Green)	1321	F																				
	4856	F				X																
	2282	M				X																
	2041	M				X																
	8356	M																				
	0127	M																				
	0515	F		X																		
	2354	M				X																
	6001	M				X																
	9002	M																				
	4065	F			X																	
	4009	M																				
	5012	M					X															
	3021	M																				
	0628	F											X									
	5335	F					X															
	3327	F																				
	7768	F			X																	
	6810	F			X																	
	9616	F																				
	0070	F				X																
	1564	F		X																		
	3569	M																				
	4793	M									X											
Controls Group #3 (Blue)	1347	F					X															
	2878	M		X																		
	4083	M		X																		
	9261	F		X																		

WRAMC FORM 111
15 JAN 1954

(b)(6) [redacted]

Bruce Ivins - 17Dec02

After 21 days

LABORATORY WORK COPY		DATE 29 OCT 02
TEST TITLE B01-11	DOSE 0.2ml	NOTEBOOK
Ivins (Bact. Div)	ROUTE SQ	NUMBER 1
ANIMAL Rb	Rx	PAGE 1
		SHEET

	MONTH		OCT		NOV										NOV						NOV			
	DATE		30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	DAYS AFTER INOCULATION		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Group #1																							
464	5116	M																						
468	1268	F																						
FT 479	4319	M		1																				
472	3854	F																						
478	6276	M			1																			
465	7597	F																						
466	9783	F																						
469	5076	F																						
471	0881	M																						
473	8324	M																						
483	1888	M				1																		
480	8554	M																						
462	2830	F																						
463	2859	F																						
485	2124	F		1																				
467	7576	F																						
474	1302	F					1																	
484	0834	F																						
481	6531	M				1																		
482	3038	M																						
475	5871	M							1															
470	9351	F										1												
477	4065	M			1																			
476	0028	M		1																				

WRAMC FORM 111
15 JAN 1954

Bruce Ivins - 17 Dec 02

After 21 days

LABORATORY WORK COPY

DATE 29 Oct 02

TEST TITLE B01-11

DOSE 0.2ml

Ivins (Bact. Div)

ROUTE SQ

ANIMAL Rb

Rx

NOTEBOOK
NUMBER 1
PAGE 2
SHEET

	MONTH																					
	DATE	30	31	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20
	DAYS AFTER INOCULATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Group #2																						
490	1321 F																						
493	4856 F				1																		
503	2282 M				1																		
508	2041 M				1																		
505	8356 M																						
507	0127 M																						
500	0515 F		1																				
513	2354 M				1																		
504	6001 M				1																		
510	9002 M																						
497	4065 F			1																			
509	4009 M																						
502	5012 M					1																	
506	3021 M																						
501	0628 F											1											
492	5335 F					1																	
494	3327 F																						
498	7768 F			1																			
491	6810 F			1																			
496	9616 F																						
495	0070 F				1																		
499	1564 F		1																				
512	3569 M																						
511	4793 M									1													
	Group #3																						
487	1347 F					1																	
488	2878 M		1																				
489	4083 M		1																				
486	9261 F		1																				

WRAMC FORM 111
13 JAN 1954

1

Brucellosis - 17 Dec 02

For (b)(6) [redacted] - Fall of 2003

AVA-FAV047 8 sepol

20 doses of rPA/Alhydrogel at 50ug/ml

lot 100506 = rPA

8.212 ml PBS
0.848 ml rPA
0.94 ml Alhydrogel

File 279A-WF-222936-1B, sec 4