

VEB-1 (298) 1  
February 5, 1990

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, GA 30601

Dear (b)(6)

The field safety trial data for Rabies Vaccine, (b)(4) (b)(4) are satisfactory. There was one adverse reaction reported in over 2,951 animals vaccinated. It is understood that additional data will be submitted when reports are sent to you by practicing veterinarians.

The Outline of Production change to include vaccination of ferrets has been approved.

Labels or sketches have been approved and processed.

Sincerely,

Robert B. Miller  
Senior Staff Veterinarian  
Veterinary Biologics  
Biotechnology, Biologics,  
and Environmental Protection

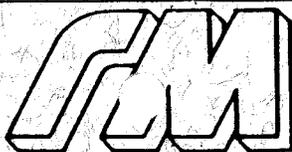
cc:

NVSL, Ames, IA (w/cy of incom)  
VBFO, Ames, IA (w/cy of incom)

APHIS:BBEP:RBMiller:cs:436-8674:2/5/90:rmi.2a



RMI.2A



115 Transtech Drive  
Athens, Georgia 30601 USA

**RHONE MERIEUX, INC.** Tel. (404) 548-9292, Facsimile (404) 548-0608

**JAN 17 1990**

15 January 1990

Dr. Robert Miller  
USDA, APHIS  
Federal Bldg., Room 838  
Vet. Biologics  
6505 Belcrest Rd.  
Hyattsville, MD 20782

Re: Rabies Vaccine, Killed Virus, Product Code (b)(4)

Dear Dr. Miller:

We have enclosed a Ferret Field Trial For Safety Report conducted at 2 farms and 1 private practice for the above products. A total of 2,951 ferrets were vaccinated. We request that this study meet the requirements for field safety. As you recall from our protocol and conversations, this report covers all requirements for commercial farm and one of the four requested private practices. At a later time the remaining 3 private practice field trials, with an approximate total of 75 additional vaccinated ferrets, will be submitted.

The only remaining requirement before the ferret claim approval is the final labeling. This we hope to submit in the next few weeks.

Sincerely,

(b)(6)

R&D/Regulatory Manager

Route: (b)(6)

BP:ss

(b)(4)

LET  
1/29/60

**RABIES VACCINE, KILLED VIRUS**

Subject: Field trial for safety in ferrets.

Procedure: Ferrets at two farms and one private practice totaling 2,951, were vaccinated subcutaneously with a one ml dose, once at 12 weeks of age or older. Animals were observed for local and systemic reactions due to vaccination over a 14 day period.

Conclusion: The vaccine was proven safe under field conditions. Out of 2,951 vaccinated ferrets only one allergic reaction was noted and no significant local reactions occurred.

Investigator:

(b)(6)

R&D/Regulatory Manager

Reviewed by:

(b)(6)

Acting Directory R&D

(b)(6)

Asst. General Manager

BP:ss

(b)(4)

**RABIES VACCINE FOR FERRETS SAFETY FIELD TRIAL****I. PURPOSE**

To evaluate the safety of our current licensed inactivated rabies vaccine in ferrets.

**II. MATERIALS****A. Vaccine**

Released serials (b)(4) of Rabies Vaccine were used throughout the trial. The vaccine consisted of inactivated rabies virus with an (b)(4) (b)(4) produced according to the current outline of production for (b)(4) (b)(4)

**B. Animals**

Ferrets from two commercial farms and pet ferrets in one private practice were used in this study. Both farms housed their ferrets in enclosed buildings in gang cages.

**III. PROCEDURES****A. Vaccination**

Ferrets were vaccinated with one ml, one dose (single vaccination) at 12 weeks of age or older. All vaccinations were done subcutaneously in the upper neck region.

1. Two thousand, seven hundred and ten ferrets were vaccinated at Pathvalley Farms in Willow Hill, PA by Dr. Robert Owen.
2. Two hundred ferrets were vaccinated at (b)(4) in (b)(4) by (b)(6)
3. Forty-one ferrets were vaccinated in a private practice by (b)(6) in (b)(6)

**B. POST VACCINATION EVALUATION**

All vaccinated ferrets were observed for 14 days. Any reactions associated with vaccination, local or systemic, were recorded by the attending veterinarian. See Appendices A, B and C.

**IV. RESULTS****A. Systemic Reactions**

The only one adverse reaction was noted within one hour of vaccination. Clinical signs included severe dyspnea and disorientation. The ferret recovered after administration of epinephrine.

**B. Local Reactions**

All ferrets vaccinated on the farms were checked several times during the 14 day observation with no significant swellings or abscesses. Upon close examination and palpitation, approximately (b)(4) the ferrets at (b)(6) and (b)(4) the ferrets at (b)(6) (b)(6) had mild thickening in the subcutaneous tissue at or near the vaccination site. All of these mild tissue reactions disappeared by the 14<sup>th</sup> day post vaccination.

**V. CONCLUSIONS**

The vaccine was shown to be safe under field conditions. Out of 2,951 vaccinated ferrets only one allergic reaction was noted and no significant local reactions occurred other than the mild, transient thickening in the subcutaneous tissue which is common and expected with this type of vaccine.

**APPENDIX A**

(b)(4)

(b)(6)

Date: December 18, 1989

To: (b)(6)

From: (b)(6)

Re: Report of Safety Field Trial for Rabies Vaccine for Ferrets

This trial was initiated on (b)(4) in (b)(4). On this date, we vaccinated a total of 2,710 ferrets by administering 1 ml of (b)(4) in the dorsum of the neck. Of this 2,710 animals, 750 were from 3 months to 1 year of age, 1,420 were from 1 year to 2 years of age, and 540 were over 2 years of age.

The only adverse reaction noted on the day of administration was an allergic reaction in one animal. Clinical signs included (b)(4). (b)(4) The animal recovered after the administration of (b)(4).

Animals were checked daily for adverse reactions such as swelling or abscessation. None were noted. I personally palpated a representative sample of vaccinated animals six days post injection and found that approximately (b)(4) of the animals had some mild (b)(4) at the injection site. This reaction was no longer palpable when the animals were checked at thirteen days post injection.

Death loss in the colony for this two week period was at or below my normal expectations. For that period, I performed necropsies on (b)(4) and found only normal causes of mortality in the colony. None of these deaths were, in my opinion, vaccine related.

Other parameters which I use to measure the status of the colony such as litter size, abortion rate, and pregnancy rate, all remained within normal limits.

Overall, I saw no change in the status of the colony as a result of the administration of this vaccine. The animals exhibited no untoward reactions during vaccine administration and the parameters which I use to assess the health status of the colony all remained within normal limits.

Submitted by:

(b)(4)

**APPENDIX B**

(b)(4)

(b)(6)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS - (14 DAY OBSERVATION)
1	1		11/16/89	NONE
2				
3				
4				
5				
6				
7				
8				
9				
10				Small sample sized syringing at injection site - gone at 14 days.
11				NONE
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25	25			

CONDUCTED BY

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)

(b)(4)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	OWNER (b)(4)	IML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	26		11/16/89	None
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				slight swelling (less than previous) at injection site - gone 14 days None
21				
22				
23				
24				
25	50			

CONDUCTED BY

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)

(b)(4)

FERRETT RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	51		11/16/89	NO. NR
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25	75			

CONDUCTED BY

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)  
(b)(4)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	IML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	76		11/16/89	None
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24	↓			
25	100		↓	

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)  
(b)(4)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	101		11/16/89	NONE
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				See record swelling at injection site - gone after 14 days.
14				NONE
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25	125			

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)

(b)(4)

FERRETT RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	126		11/16/89	NONE
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				small muscle-piped swelling at injection site gone by day 14
21				NONE
22				
23				
24				
25	150			

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)

(b)(4)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	151		11/16/89	None
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25	175			

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)  
(b)(4)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	(b)(4)	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	170		11/16/89	NONE
2				
3				
4				Pen- and swelling at injection site - gone at 14 days
5				NONE
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25	200			

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)  
(b)(4)

**APPENDIX C**

(b)(4)

(b)(6)

FERRET RABIES VACCINE (IMRAB™) FIELD TRIAL

#	IDENTIFICATION	OWNER	IML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	"OSCAR"	(b)(6)	12-1-89	No reaction
2	"BOBY"		12-1-89	No reaction
3	Apple sauce		12-2-89	NO reaction
4	Slinkie	" "	" "	NO reaction
5	Smudgeon	" "	" "	NO reaction
6	Bramie	" "	" "	NO reaction
7	Little one	" "	" "	NO reaction
8	Maggie	" "	" "	NO reaction
9	Marsel	" "	" "	NO reaction
10	Flash	" "	" "	NO reaction
11	Natasha	(b)(6)	12-2-89	} Owner has not phoned in any reaction } we could not reach him by phone
12	Bowie	" "	" "	
13	Popsy	(b)(6)	12-2-89	<del>NO</del> NO reaction
14	NATHAN	" "	" "	NO reaction
15	FERRET #1	" "	" "	NO reaction
16	" 2	" "	" "	NO reaction
17	" 3	" "	" "	NO reaction
18	" 4	" "	" "	NO reaction
19	" 5	" "	" "	NO reaction
20	" 6	" "	" "	NO reaction
21	" 7	" "	" "	NO reaction
22	" 8	" "	" "	NO reaction
23	" 9	" "	" "	NO reaction
24	" 10	" "	" "	NO reaction
25	" 11	" "	" "	NO reaction

CONDUCTED BY:

(b)(6)  
SIGNATURE

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)  
(b)(4)

FERRET RABIES VACCINE (IMRAB<sup>SM</sup>) FIELD TRIAL

#	IDENTIFICATION	OWNER	1ML DOSE SQ DATE VACCINATED	REACTIONS (14 DAY OBSERVATION)
1	Ferret # 12	(b)(6)	12-2-89	NO reaction
2	" 13	" "	"	NO reaction
3	" 14	" "	"	NO reaction
4	" 15	" "	"	NO reaction
5	" 16	" "	"	NO reaction
6	" 17	" "	"	NO reaction
7	" 18	" "	"	NO reaction
8	" 19	" "	"	NO reaction
9	" 20	" "	"	NO reaction
10	" 21	" "	"	NO reaction
11	" 22	" "	"	NO reaction
12	Luther	(b)(6)	12-7-89	NO reaction
13	Fancy	(b)(6)	12-7-89	NO reaction
14	SHAWN	(b)(6)	12-9-89	NO reaction
15	SANDY	(b)(6)	12-9-89	NO reaction
16	FERT	(b)(6)	12-9-89	No reaction
17				
18				
19				
20				
21				
22				
23				
24				
25				

CONDUCTED BY:

(b)(6)

PRINTED NAME:  
CLINIC ADDRESS:

(b)(6)

(b)(4)

V&A (298) 1  
December 6, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, GA 30601

Dear Dr. Pitts:

This is in response to your submissions dated (b)(4)  
(b)(4) regarding a ferret claim for your Rabies Vaccines, (b)(4)  
(b)(4)

The report of efficacy testing in ferrets is satisfactory for the purpose submitted. As we have previously discussed, there was a deviation from the protocol in that no data was submitted on tests for rabies virus in saliva and for rabies antibody in the cerebrospinal fluid (CSF).

I understand that some samples of saliva and CSF were collected during the trial for later analysis. This should be completed as soon as possible in order to increase the general knowledge about rabies in ferrets. The lack of this information will not delay licensing.

This is to confirm that you have been authorized to conduct field safety trials in the States of (b)(4)  
(b)(4) from which letters of authorization were included in your submissions. I have also received a copy of a letter from (b)(6) authorizing you to conduct a trial in (b)(4). You are also authorized to conduct the trial in that State.

The experimental label is satisfactory. A date-stamped copy is enclosed for your records.

This authorization expires on November 30, 1990. If additional time is needed to complete the study, an interim report of progress must accompany your request for an extension.

Sincerely,

Robert B. Miller  
Senior Staff Veterinarian  
Veterinary Biologics  
Biotechnology, Biologics,  
and Environmental Protection

Enclosures

cc:  
NVSL, Ames, IA (w/cy of incom)  
VBFO, Ames, IA (w/cy of incom)  
R. Harrington, Jr., CR/RD, Ft. Worth, TX  
W. W. Buisch, NR/RD, Scotia, NY

APHIS:BBEP:RBMiller:ts:68674:12-01-89:RMI.11C  
JP Dae



115 Transtech Drive  
Athens, Georgia 30601 USA

ONE MERIEUX, INC. Tel. (404) 548-9292, Facsimile (404) 548-0608

29 September 1989

OCT 02 1989

Dr. Robert Miller  
USDA/APHIS  
Federal Building, Room 838  
Veterinary Biologics  
6505 Belcrest Road  
Hyattsville, MD 20782

Re: Rabies Vaccine, KV, Ferret Field Trial

Dear Dr. Miller:

We request permission to conduct a field trial for the above product in ferrets in order to partially satisfy requirements for an additional species label claim. This request is in accordance with 9CFR 103.3.

Included in this submission are:

1. One copy of a letter of permission from the states of (b)(4)
2. Two copies of a tentative list of names of the proposed recipients and quantities of vaccine to be shipped to each individual.
3. Two copies of a description of the product and recommendations for use.
4. Three copies of an experimental label.
5. Two copies of a proposed protocol of investigation.
6. Four copies of a 2008 on Serial 12041. This serial is a routine release product.

We would appreciate your attention to this matter at your earliest convenience.

Sincerely,

(b)(6)

R&D/Regulatory Manager

Route: (b)(6)

BP:ss

ferret (b)(4)



STATE OF NEW YORK  
DEPARTMENT OF AGRICULTURE AND MARKETS  
1 WINNERS CIRCLE - CAPITAL PLAZA  
ALBANY, NEW YORK 12235

DIVISION OF ANIMAL INDUSTRY  
518 457-3502  
518 457-4187

BUREAU OF DOG LICENSING  
518 457-2728

August 16, 1989

(b)(6)

R & D/Regulatory Manager  
Rhone Merieux Inc.  
115 Transtech Drive  
Athens, Georgia 30601

Dear

(b)(6)

In response to your letter dated August 7, 1989, please consider this letter approval to conduct a small field trial with an inactivated Rabies Vaccine (b)(4) on domestic ferrets.

It is understood that this department assumes no responsibility for any adverse reaction that might occur following the use of this product.

Very truly yours,

Bruce Widger, DVM  
Director

W:RE:DK



COMMONWEALTH OF PENNSYLVANIA  
DEPARTMENT OF AGRICULTURE  
2301 N. CAMERON STREET, HARRISBURG, PA 17110-9408

September 26, 1989

BUREAU OF ANIMAL INDUSTRY  
MAX A. VAN BUSKIRK, JR., V.M.D.  
DIRECTOR  
TELEPHONE: (717) 783-5301

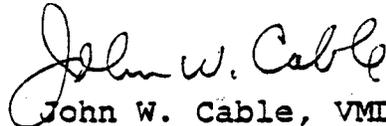
(b)(6)

R&D/Regulatory Manager  
Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, GA 30601

Dear Mr. Pitts:

Permission is granted to Rhone Merieux, Inc. to field test  
(b)(4) domestic ferrets in Pennsylvania. The field test must  
be conducted in accordance with the USDA approval field test  
protocol for (b)(4) ferrets.

Sincerely,

  
John W. Cable, VMD, MS



STATE OF MINNESOTA

BOARD OF ANIMAL HEALTH

160 AGRICULTURE BLDG.

90 W. PLATO BLVD.

ST. PAUL, MN 55107

(612) 296-2942

August 14, 1989

(b)(6)

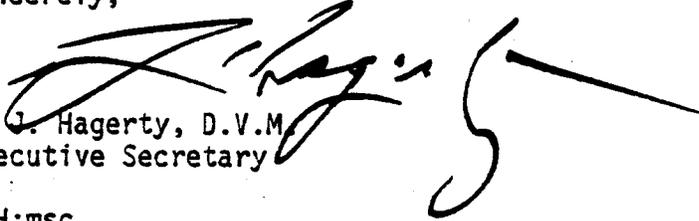
R&D/Regulatory Manager  
Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, Georgia 30601

Dear (b)(6)

Permission is hereby granted to Rhone Merieux, Inc., of Athens, Georgia to conduct a field trial with (b)(4) inactivated rabies vaccine, in domestic ferrets in Minnesota. Permission is granted under the following conditions:

1. Field trials shall be carried out in accordance with CFR, Title 9, Chapter 1, Part 103, Section 103.3.
2. One copy of a tentative list of the names of the proposed recipients and quantity of products as outlined in 103.3 (b) shall be mailed to this office, as well as subsequent changes.
3. This permit will expire August 14, 1990.

Sincerely,

  
T. J. Hagerty, D.V.M.  
Executive Secretary

TJH:msc

# Iowa Department of Agriculture and Land Stewardship

DALE M. COCHRAN  
SECRETARY OF AGRICULTURE



SHIRLEY DANSKIN-WHITE  
DEPUTY SECRETARY OF AGRICULTURE

HENRY A. WALLACE BUILDING  
DES MOINES, IOWA 50319

August 21, 1989

Rhone Merieux, Inc.

(b)(6)

R & D/Regulatory Manager  
115 Transtech Drive  
Athens, GA 30601

Dear

(b)(6)

You are hereby authorized to conduct a small field  
trial in Iowa involving domestic ferrets with your

(b)(4)

Conformance with 9CFR 103.3 is necessary.

Sincerely,

Walter D. Felker, D.V.M.  
State Veterinarian

WDF/bs

19 September 1989

RABIES VACCINE FOR FERRETS  
SAFETY FIELD TRIAL PROTOCOL

I. PURPOSE:

To evaluate the safety of our current licensed inactivated rabies vaccine in ferrets.

II. MATERIALS:

A. VACCINE:

A released serial of Rabies Vaccine produced according to the current outline of production will be used throughout the trial. The vaccine consists of inactivated rabies virus with an (b)(4) produced according to the current outline of production for product code (b)(4) (b)(4)

B. ANIMALS:

At least 400 ferrets will be vaccinated; at a minimum of 2 separate locations (200 ferrets each). All ferrets will be 12 weeks of age or older and in good health at time of vaccination.

III. PROCEDURES:

A. VACCINATION:

Ferrets will be vaccinated with one ml, one dose (single vaccination) at 12 weeks of age or older. All vaccinations will be done subcutaneously in the upper neck region.

B. POST VACCINATION EVALUATION:

All vaccinated ferrets will be observed for 14 days. Any reactions associated with vaccination, local or systemic, will be recorded.

C. REPORT OF TRIAL:

All data from the trial will be forwarded to Rhone Merieux, Inc. for evaluation and reporting to USDA.

BP:ss

(b)(4)

OCT 02 1989

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

VETERINARY BIOLOGICS PRODUCTION AND TEST REPORT

NOTE: Submit an original and one copy for every serial or subserial which reaches any stage of identification and testing

3. MAILING ADDRESS OF LICENSEE OR PERMITTEE (Include ZIP code)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, Ga 30601

1. PAGE 1 OF 1	2. LICENSE OR PERMIT NO. 298
4. FILL DATE 05/04/89	5. PRODUCT CODE NO. (b)(5)
6. EXPIRATION DATE (b)(4)	7. SERIAL OR SUBSERIAL NO. (b)(4)

8. TRUE NAME OF PRODUCT

Rabies Vaccine, Killed Virus

9. TEST DATA (For additional test data, use VS Form 14-8A)

TEST REFERENCE A	TEST DATES		RESULTS D	INSERT CODE		E
	STARTED B	CONCLUDED C		S - SATISFACTORY I - INCONCLUSIVE	U - UNSATISFACTORY NT - NO TEST	
(b)(4) 1)	(b)(4)	(b)(4)	Sterility Test:			S
2)			10/10 No growth FTM 30-35°C 1) NT* 2)			
(b)(4)	(b)(4)	(b)(4)	10/10 No growth FTM 20-25°C			S
(b)(4)			Mouse Safety: 8/8 mice survived			
(b)(4)			Guinea Pig Safety: 2/2 guinea pigs well			
(b)(4)			Rabies potency - NIH (KV)			
(b)(4)	(b)(4)	(b)(4)	Relative potency EPD50 vaccine EPD50 reference LD50 challenge			S
(b)(4)			Rabies inactivation, rabbits (KV): 2/2 rabbits well			
(b)(4)			Rabies inactivation, mice (KV): 20/20 mice well			
(b)(4)			Rabies inactivation, most susc. species species: dogs 3/3 animals well			

10. INVENTORY FOR RELEASE (Use a separate line for each size container)

NO. OF CONTAINERS A	CONTAINER SIZE (DOSES, ML. OR UNITS) B	TOTAL DOSES, ML. OR UNITS C
49,295	(b)(4)	(b)(4)
<b>TOTAL</b>		<b>TOTAL</b>
49,295		(b)(4)

REC'D VBFO JUN 16 '89  
11. REMARKS

(b)(4) Samples sent to Ames 05/09/89  
\*Media prepared incorrectly

12. DISPOSITION BY FIRM

- ELIGIBLE FOR RELEASE  
  DESTROYED  
  TO BE REPROCESSED AND RETESTED  
 OTHER (Explain)

13. SIGNATURE (Authorized Firm Representative)

(b)(6)

14. TITLE

Quality Assurance Mgr

15. DATE

06/09/89

16. DISPOSITION BY APHIS

- NOT TO BE TESTED  
  TESTS COMPLETED, SATISFACTORY  
 TESTS COMPLETED, UNSATISFACTORY (Explain)  
  OTHER (Explain)

17. SERIAL

(b)(6)

18. TITLE

BIOLOGICS SPECIALIST

19. DATE

JUN 19 '89



115 Transtech Drive  
Athens, Georgia 30601 USA

NOV 24 1989

**RHONE MERIEUX, INC.** Tel. (404) 548-9292. Facsimile (404) 548-0608

21 November 1989

Dr. Robert Miller  
USDA/APHIS  
Federal Building, Room 838  
Veterinary Biologics  
6505 Belcrest Road  
Hyattsville, MD 20782

Re: Rabies Vaccine, Killed Virus, Product [REDACTED]  
Ferret Field Trial

(b)(4)

Dear Dr. Miller:

We request the addition of four (4) more states and the following investigators to the list already approved for field trial testing. These states are:

Illinois - [REDACTED]  
Maryland - [REDACTED]  
Virginia - [REDACTED]  
Wisconsin - [REDACTED]

(b)(6)

(b)(4)

The above practitioners will be vaccinating a minimum of 25 ferrets and will give their owner clients instructions that if there are any post vaccination reactions to notify them.

Previously approved were the states of New York, Pennsylvania, Iowa, Minnesota and Texas.

Thank you for your continued cooperation in this matter.

Sincerely,

[REDACTED]  
(b)(6)

R&D/Regulatory Manager

Route: [REDACTED]

(b)(6)

BP:ss

[REDACTED]  
(b)(4)



State of Illinois

NOV 24 1989

# DEPARTMENT OF AGRICULTURE

Division of Animal Industries

State Fairgrounds / P.O. Box 19281 / Springfield 62794-9281

Administrative Unit  
(217) 782-4944

Bureau of Animal Health  
(217) 782-4944

Bureau of Animal Welfare  
(217) 782-6657

Bureau of Meat and Poultry Inspection  
(217) 782-6684

Bureau of Compliance and Enforcement  
(217) 785-4709

November 14, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, GA 30601

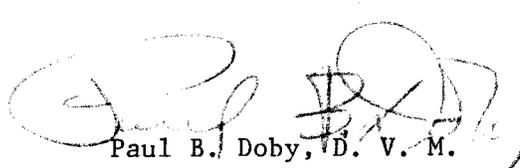
Dear (b)(6)

This is grant permission for Rhone Merieux, Inc. to conduct field trials in Illinois with an inactivated Rabies Vaccine in domestic ferrets.

This permit in no way involves the Illinois Department of Agriculture or me with any liability relative to the use of this product in Illinois.

Very truly yours,

DIVISION OF ANIMAL INDUSTRIES

  
Paul B. Doby, D. V. M.  
Superintendent

PBD:vfk



William Donald Schaefer  
Governor

Melvin A. Steinberg  
Lt. Governor

Wayne A. Cawley, Jr.  
Secretary

Robert L. Walker  
Deputy Secretary

STATE OF MARYLAND  
DEPARTMENT OF AGRICULTURE  
Animal Health Section

November 15, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, Georgia 30601

Dear (b)(6)

Authorization is granted to Rhone Merieux, Inc. to conduct a small field trial in the state of Maryland with (b)(4) inactivated Rabies Vaccine in domestic ferrets.

This authorization is contingent on tests being conducted in accordance with applicable federal regulations and providing us with a summary of the test results when completed.

Sincerely,

Henry A. Virts, D.V.M.  
State Veterinarian

HAV:lg

50 HARRY S TRUMAN PARKWAY, ANNAPOLIS, MARYLAND 21401

(301) 841-5700  
Baltimore/Annapolis Area



(301) 261-8106  
Washington Metro Area

NOV 24 1989



# COMMONWEALTH of VIRGINIA

S. MASON CARBAUGH  
Commissioner

DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES  
DIVISION OF ANIMAL HEALTH  
WASHINGTON BUILDING, SUITE 600  
1100 BANK STREET, RICHMOND, VA 23219

WILLIAM D. MILLER, D.V.M.  
State Veterinarian

November 14, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, Georgia 30601

RE: (b)(4) Inactivated Rabies  
Vaccine), (Ferrets)

CORRESPONDENCE: November 6, 1989

Dear (b)(6)

We have reviewed your request to field-test the above-referenced product in the Commonwealth of Virginia and have approved such testing of that product, subject to the following conditions:

- (1) That the testing of the product shall cease immediately upon the withdrawal, cancellation, revocation, suspension, termination, or nullification of the approval of the Biologics Division, Animal and Plant Health Inspection Service, United States Department of Agriculture, to ship the product in order to conduct the field test;
- (2) That you provide us with the name of the investigator and the precise address of the place where the field trial is to be conducted prior to commencing the field trial;
- (3) That you immediately report to this office any adverse reaction to this product, wherever it occurs; and

(b)(6)

Rhone Merieux, Inc.  
November 14, 1989  
Page Two

- (4) That you provide this office upon completion of the final test a summary of your results.

If this office can be of further assistance to you, please do not hesitate to contact us.

Sincerely,

*William D. Miller*

William D. Miller, D.V.M.  
State Veterinarian

WDM/slj



State of Wisconsin

NOV 24 1989

Department of Agriculture, Trade & Consumer Protection

310 N. Midvale Boulevard  
Madison, WI 53705

Howard C. Richards  
Secretary

November 14, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, GA 30601

Dear (b)(6)

Permission is granted for Rhone Merieux, Inc., Athens, GA, to conduct field studies with the following experimental vaccine in Wisconsin:

(b)(4) - an inactivated Rabies Vaccine in domestic ferrets

Permission is granted provided that the field trials will be conducted in accordance with 9CFR Part 103 which regulates experimental biologics in state of development.

Dr. Dennis Carr has been named State Veterinarian for the State of Wisconsin. Please update your records.

Sincerely,

  
Dennis J. Carr, D.V.M.  
State Veterinarian  
ANIMAL HEALTH DIVISION

DJC/cy

1/vacperm/djc/l

# Texas Animal Health Commission

an equal opportunity employer

210 Barton Springs Road  
P.O. Box 12966  
Austin, Texas 78711-2966

W. Holcombe, DVM  
Executive Director

512/479-6697



NOV 22 1989

*Dr. Miller*

## COMMISSIONERS:

John Cargile,  
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Joe Hathoot

Allan C. Oltjen, DVM

James B. Owen

Florence Rieck

Bruce Rigler

James D. Sartwell

Gaye L. Seawright

James Snyder

Mary Nan West

November 10, 1989

(b)(6)

Rhone Merieux, Inc.  
115 Transtech Drive  
Athens, Georgia 30601

Re: Inactivated Rabies Vaccine for use in  
domestic ferrets - (b)(4)

Dear (b)(6)

This letter will serve as authorization from the State of Texas for you to conduct clinical field trials using the above referenced vaccine.

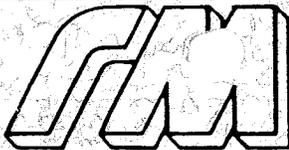
This authorization is given subject to your complying with the requirements of USDA Veterinary Biologics regulations, 9 CFR Section 103.1, .2 and .3.

Very truly yours,

*John W. Holcombe*  
John W. Holcombe, DVM  
Executive Director

JWH:jac

cc: Dr. David Espeseth ✓



115 Transtech Drive  
Athens, Georgia 30601 USA

**RHONE MERIEUX, INC.** Tel. (404) 548-9292, Facsimile (404) 548-0608

16 November 1989

Dr. Robert Miller  
USDA/APHIS  
Federal Building, Room 838  
Veterinary Biologics  
6505 Belcrest Road  
Hyattsville, MD 20782

Re: Rabies Vaccine, Killed Virus, Product Cod (b)(4) Ferret Claim

Dear Dr. Miller:

Enclosed are three copies of the ferret rabies efficacy report. These results meet the requirements of 9CFR 113.129(b). Our final labeling and field trial results will be submitted in the near future.

We would appreciate your review and approval of this efficacy report at your earliest convenience.

Sincerely,

(b)(6)

Route:

(b)(6)

BP:ss

(b)(4)

16 November 1989

ST  
11/22/89

PROTECTION OF DOMESTIC FERRETS (MUSTELA PUTORIUS FURO) WITH  
INACTIVATED RABIES VIRUS VACCINE

SUMMARY

Efficacy of a subcutaneously-administered vaccine for prevention of rabies was evaluated in the domestic ferret. Ferret immunity was challenged by the (b)(4) of street rabies virus. All ferrets developed titers of rabies virus-neutralizing antibodies within 30 days of vaccination (b)(4) that were maintained for at least one year (b)(4) as compared to no (b)(4) challenge. (b)(4) Following rabies virus challenge, (b)(4) vaccinated ferrets survived versus more than (b)(4) in control ferrets. These results demonstrate the protective efficacy of a commercial inactivated rabies vaccine of at least one year duration for the domestic ferret.

Supported in part by the generosity of (b)(4) The investigators thank (b)(6) and (b)(6) technical or logistical assistance.

INVESTIGATORS:

(b)(6)

REVIEWED BY:

(b)(6)

## INTRODUCTION

Based upon increasing annual rates of commercial ferret sales and rising membership statistics provided by national associations, domestic ferrets (Mustela putorius furo) are gaining in popularity as pets.<sup>1-3</sup> For example, from solicited state surveys the California Domestic Ferret Association estimates that there are currently some five - seven million pet ferrets in approximately four - five million households nationwide. More accurate population census data are currently unavailable. As pet ferret numbers increase, public health authorities are faced with mounting concern over the threat of rabies exposure to humans resulting from ferret bites.<sup>4</sup> Although there are no approved commercial rabies vaccines for ferrets in the United States, and no published information on the effectiveness of existing canine or feline rabies vaccines in preventing lethal rabies virus infection in ferrets, the routine use of inactivated rabies vaccine at three to six months of age, repeated annually, is commonly suggested to veterinary practitioners<sup>5-7</sup>. Modified-live rabies vaccines are strongly discouraged because of the increased potential risk of vaccine-induced rabies. To gain USDA approval, controlled laboratory data must be obtained concerning the duration of immunity and efficacy conferred by a given rabies vaccine against a virulent street rabies virus challenge. We report here the first serologic and protective responses of domestic ferrets to a commercial inactivated rabies virus vaccine over a one year course of study.

## MATERIALS AND METHODS

Vaccine - The vaccine consisted of liquid inactivated rabies virus (b)(4) diluted to a minimum NIH potency value and was the same commercial rabies vaccine<sup>a</sup> approved by USDA for feline, canine, equine, bovine, ovine, and used to conduct minimal rabies vaccine dose efficacy in the feline and canine. The identity of the specific vaccine was designated (b)(4). The mean of (b)(4) NIH values in mice, tested prior to ferret vaccination was (b)(4) (Table 3).

Challenge virus - The rabies challenge consisted of street virus of fox origin<sup>a</sup> having a minimum concentration of (b)(4) (b)(4)

Animals - Ferrets used as vaccinates and controls initially consisted of 90 clinically normal 12-week-old domestic ferrets<sup>c</sup> (20 males, 70 females) housed inside environmentally-controlled production buildings and were maintained on a commercial dry diet and water provided ad libitum. All ferrets were neutered and descented by surgical removal of the anal scent glands prior to study. Ferrets were identified by metal ear tags and a permanent body tattoo. Ferrets that succumbed over the year of study prior to rabies virus challenge (i. e., due to bleeding, shipment, etc.) were not replaced.

Serological assay - Serum virus neutralization titers were conducted on serum samples of all ferrets before vaccination and at (b)(4) months post-vaccination.

Rabies neutralizing antibody levels were determined by the (b)(4) (b)(4). The test was adapted so that microtitration equipment could be used. The cell line used was (b)(4) and the rabies virus strain was (b)(4)

-----  
(b)(4) Rhone Merieux, Inc., Athens, Georgia  
<sup>b</sup> Obtained from the National Veterinary Services Laboratory, Ames, Iowa  
<sup>c</sup> Donated by (b)(4)

The VNA titer was recorded as the reciprocal of the highest serum dilution that resulted in a (b)(4) in vitro. Seroconversion was considered on the basis of at least a (b)(4) in titer between paired sera. The (b)(4) was calculated from (b)(4) transformed data, which was then retransformed to a standard titer format.

Experimental Protocol - Complete physical examinations were conducted on all ferrets prior to inclusion in the study. Pre-vaccination (Day 0) blood samples were obtained via (b)(4)

(b)(4) days post-vaccination. On Day 0 (b)(4) Forty-five ferrets were vaccinated with 1.0ml of inactivated rabies virus vaccine administered subcutaneously. Forty-five ferrets were held as unvaccinated controls. At (b)(4) post-vaccination, all surviving ferrets were transferred to isolation facilities for challenge of immunity. Animals were housed in strict isolation in individual stainless-steel squeeze cages and were provided with food and water ad libitum. Due to limited challenge facilities the vaccination and controls were divided randomly into three separate challenge groups and challenged approximately (b)(4) apart. The first challenge group consisted of (b)(4). The second challenge group consisted of (b)(4). The third challenge group consisted of (b)(4). The three challenge dates were (b)(4). Prior to handling, ferrets were sedated by the (b)(4) at approximately (b)(4). Once sedated, ferrets were inoculated with (b)(4) street rabies virus in each masseter. Ferrets were observed daily and were sedated and euthanized by (b)(4) overdose at the first definitive clinical signs characteristic of rabies virus infection. Rabies virus diagnosis was confirmed by routine (b)(4) performed upon brain impressions. All survivors were similarly euthanized and examined (b)(4)

-----

(b)(4)  
(b)(4)

## RESULTS

After preliminary physical examination, pre-vaccination bleeding, random group assignment, or initial vaccination of the original 90 ferrets, non-specific deaths left only (b)(4) available as vaccinates and (b)(4). By one year following vaccination, (b)(4) ferrets had died (due to handling, bleeding, etc.) from the vaccinated group and (b)(4) the control group. All vaccinated ferrets developed rabies VNA in the (b)(4) following vaccination (Table 1). Rabies VNA titers generally peaked by (b)(4) and slowly declined over the course of the year. In contrast, no rabies VNA were detected at any time in the control group (Table 2).

Following street rabies virus inoculation one year post-vaccination, (b)(4) survived compared to (b)(4) which succumbed to rabies, (b)(4) following challenge (Table 1 and 2). All rabies-suspect ferrets were confirmed as rabies virus positive by the (b)(4) (b)(4) upon brain impressions; all survivors were negative. Neither of the two surviving control ferrets demonstrated prior rabies VNA or clinical signs suggestive of rabies. The four vaccinates that succumbed to rabies had existing VNA titers (b)(4) vaccination ranging from (b)(4) (b)(4) vaccinates had low VNA titers in this general range at the time of challenge, but remained healthy throughout the study.

## DISCUSSION

The results of this challenge of ferret (b)(4) demonstrate that domestic ferrets can be protected against lethal rabies virus infection for one year by the administration of a single subcutaneous dose of commercial inactivated rabies vaccine given at (b)(4). Vaccination afforded significant protection against a virulent street rabies virus challenge that resulted in the death of the clear majority of control ferrets. Explanations for deaths of the (b)(4) are only speculative. At least one of these ferrets (#4) had the poorest overall serological response of any vaccinate (Table 1). However, survival against rabies challenge may not have been solely related to the absolute VNA titer on the day of challenge. While the (b)(4) of the (b)(4) which succumbed were each below the vaccine group (b)(4) other vaccinates had comparably (b)(4) yet survived. The comparative VNA levels for vaccinates and controls were not directly measured immediately following challenge, allowing no evaluation of potential anamnestic response. Regardless, protection against lethal rabies virus infection afforded by (b)(4) is likely to be a complete process involving multiple effector mechanisms, including both humoral and cell-mediated immunity rather than by a single (b)(4). In addition, no rabies vaccine alone can be (b)(4). Acting in concert, routine boosters and effective post-exposure therapy can minimize the threat of lethal rabies virus infection.

As shown (b)(4) ferrets will develop high levels of rabies VNA in response to parenteral administration of rabies vaccine. In addition, we have demonstrated the efficacy of a commercial rabies vaccine for ferrets that provides a minimum of one year's duration of anti-rabies protection. Annual booster vaccinations would seem warranted until data generated from further laboratory research or clinical trials demonstrate efficacy in excess of one year.

## REFERENCES

1. Gorham ME. The Pet Of The Future, Small Animal Practitioner's Guide to Treating Ferrets. DVM 1985; 16:19-22.
2. Groseclose S. Horman JT. Pet Ferrets Require Special Safety Considerations. Norden News 1986; April 4:1 (Col 1-3).
3. Petzke D. The Pet Of The Year Isn't A Pinup Or S Pup, But It Is Just As Cute. Wall Street Journal 1986; april 4:1 (Col 1-3).
4. Gunley P. Can You Catch Rabies From Your Ferret? Probably not. JAMA 1981; 245:1628.
5. Ryland LM, Bernard SL, Gorham JR. A Clinical Guide To The Pet Ferret. Compend Contin Educ Pract Vet 1983; 5:25-32.
6. Randolph, RW. Preventive Medical Care For The Pet Ferret. In: Kirk, RW, ed. Current Veterinary Therapy. 9th ed. Philadelphia: WB Saunders Co., 1986; 772-774.
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8. Koprowski H. Experimental Studies On Rabies Virus. Canad J. Publ Hlth 1949; 40:60.
9. Smith JS, Yager PA, Baer GM. A Rapid Reproducible Test For Determining Rabies Neutralizing Antibody. Bull WHO 1973; 48:535-541.
10. Dean DJ, Abelseth MK. The Fluorescent Antibody Test. IN; Kaplan MM, Kowprowski H, eds. Laboratory Techniques in Rabies. Geneva: World Health Organization, 1973; 73-74.
11. Dietzschold B, Tollis M, Rupprecht CE, Celis E, Koprowski H. Angigenic Variation In Rabies And Rabies-Related Viruses: Cross-Protection Independent Of Glycoprotein-Mediated Virus-Neutralizing Antibody. J Inf Dis 1987; 156: 815-822.
12. Hoover, JP, Baldwin CA, Rupprecht CE. Serologic Response Of Domestic Ferrets (Mustela putorius furo) To Canine Distemper and Rabies Virus Vaccines. JAVMA 1989; 194: 234-238.

TABLE 1

Development of rabies (b)(4) and protective response to rabies challenge in domestic ferrets after a single subcutaneous administration of commercial inactivated virus vaccine.\*

VNA Titers (reciprocal)

	FERRET TAG#	PRE-VAC 1/11/88	30 DAY 2/11/88	90 DAY 4/11/88	180 DAY 7/12/88	270 DAY 10/12/88	365 DAY 1/11/89	RESPONSE TO CHALLENGE
1st Challenge Group	(b)(4)							Survived
								Died
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
								Survived
2nd Challenge Group	(b)(4)							Survived
								Survived
								Survived
								Survived
3rd Challenge Group	(b)(4)							Died
								Survived
								Survived
								Survived
Died prior to Challenge	(b)(4)							Died
								Died
								Survived
								Died
								Survived
								Died
								Survived

\* Ferrets were inoculated intra-masseter after rabies virus (b)(4) of street (b)(4)

TABLE 2

Rabies virus neutralizing antibody (VNA) and response to rabies challenge in nonvaccinated domestic ferrets.\*

VNA Titers (reciprocal)

	FERRET TAG#	PRE-VAC 1/11/88	30 DAY 2/11/88	90 DAY 4/11/88	180 DAY 7/12/88	270 DAY 10/12/88	365 DAY 1/11/89	RESPONSE TO CHALLENGE
1st Challenge Group	(b)(4)							Died
								Died
								Died
								Died
								Died
								Survived
								Died
								Survived
2nd Challenge Group	(b)(4)							Died
								Died
								Died
								Died
								Died
3rd Challenge Group	(b)(4)							Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
								Died
Died								
Died prior to Challenge	(b)(4)							d) -
								-
								-
								-
								-

\* Ferrets were inoculated intra-masseter after (b)(4) of street rabies virus (b)(4)



VEB 4 (298)

X 1905.20

1905.21

March 25, 1986

(b)(6)  
Merieux Laboratories, Inc.  
117 Rowe Road  
Athens, GA 30601

Dear (b)(6)

This is in response to your submission of March 7, 1986, with information to support Outline of Production changes for Rabies Vaccine, Killed Virus, Codes (b)(4)

We have reviewed the new efficacy material and the report has been filed as satisfactory to support the Relative Potency changes.

As discussed with you on March 24, 1986, we have approved and processed the revised outline for Code (b)(4). The change for Code (b)(4) will be approved upon receipt of revised labeling.

Sincerely,

G. P. Shibley  
George P. Shibley, Ph.D.  
Chief Staff Microbiologist  
Veterinary Biologics Staff  
Veterinary Services

cc:  
NVSL, Ames, IA  
VBFO, VS, Ames, IA

APHIS:VS:GPSibley:mem:436-8674:3-25-86:mem8





**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

MAR 12 1986

JST  
3/24/86

7 March 1986

Dr. George P. Shibley  
 USDA/APHIS/VS  
 Federal Building  
 6505 Belcrest Road, Room 829  
 Hyattsville, MD 20782

Dear Dr. Shibley:

Per the enclosed letter dated 7 March 1986, please find attached for your information:

- (1) Production Outline page changes and VS forms 14-15 for Product Code (b)(4) pages 12-13, Sections V.C. and VI.D.2) and Product Code (b)(4) (page 12, Section V.C.).
- (2) Reference "Laboratory Techniques in Rabies" Third Ed., Kaplan and Koprowski.
- (3) Efficacy data
  - (3.1) (b)(4) Horses, dated (b)(4)
  - (3.2) (b)(4) on Cattle, dated (b)(4)
  - (3.3) (b)(4) in Dogs, dated (b)(4)
  - (3.4) (b)(4) Sheep, reference (b)(4)
  - (3.5) (b)(4) in Cats, dated (b)(4)

Deeply appreciating your consideration, I remain:

(b)(6)

Enclosures



MAR 12 1986

JST  
3/24/86

**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

7 March 1986

Dr. George P. Shibley  
 USDA/APHIS/VS  
 Federal Building  
 6505 Belcrest Road, Room 829  
 Hyattsville, MD 20782

Dear Dr. Shibley:

RE: Rabies Vaccine, Killed Virus, Product Code (b)(4)  
 and Production Outline Changes Attached Containing Proposed  
 Changes in the Relative Potency Required at Release

We currently have two Rabies Vaccine, Killed Virus products USDA/  
 APHIS approved for production and sale in the USA. They are approved  
 as indicated below:

Table 1

Product Code	Trade Name	Species Protected	Duration of Protection	MI Dose	Relative Potency at Release
(b)(4)		Canine	1 year	(b)(4)	(b)(4)
		Feline	1 year		
(b)(4)		Canine	3 years	(b)(4)	(b)(4)
		Feline	3 years		
		Ovine	3 years		
		Bovine	1 year		
		Equine	1 year		

It is our understanding that these high Relative Potency requirements are due to high NIH values of vaccine used for (b)(4) carried out for three-year duration in cats and dogs. In 1981, new (b)(4) were initiated for these species with batch (b)(4) was demonstrated to have an NIH of 3.0. In this regard we are attaching recently completed three-year feline and canine efficacy data to support our request for a R.P. reduction. Also included is data supporting each other species for which claims are indicated.

Dr. George P. Shibley  
7 March 1986  
Page 2

Table 2  
Current Efficacy Data Summary

<u>Species</u> <sup>1</sup>	<u># Dead/# Challenged Vaccinates Controls</u>	<u>Dose Volume/ Animal</u>	<u>R.P. of Vaccine per ml</u>	<u>Months Post-vaccination at Challenge</u>
Feline	[REDACTED]			
Canine				
Ovine				
Equine				
Bovine				

<sup>1</sup> Individual reports are attached to support this data summary.

A reprint entitled [REDACTED] (b)(4) (Third Edition) Kaplan (World Health Organization), Koprowski (Wistar Institute) which describes the Relative Potency calculation procedure is also attached. This reprint demonstrates that a Relative Potency (R.P.) of [REDACTED] (b)(4) [REDACTED] (b)(4) per [REDACTED] (b)(4) i.e., the R.P. value is directly proportional to the volume of product administered.

Product Code [REDACTED] (b)(4) is recommended for use in only canine and feline species and for a duration of immunity of one year. The Table 2 data clearly supports reduction of the [REDACTED] (b)(4) release to [REDACTED] (b)(4) value provided 36 months of protection to each species tested (two years more than being recommended).

Product Code [REDACTED] (b)(4) is recommended for five species of domestic animals as detailed in Table 1. According to Table 2 the minimum R.P. value at release is most affected by the equine and ovine data. The equine trial was conducted with a [REDACTED] (b)(4) recommendation is for a [REDACTED] (b)(4) which translates into a R.P. value of [REDACTED] (b)(4) rather than the [REDACTED] (b)(4). The remaining limitation is that of the ovine data which demonstrates satisfactory protection at a [REDACTED] (b)(4) [REDACTED] (b)(4) which has been our recommended volume of product to administer per dose. We propose to change the dose volume for sheep to [REDACTED] (b)(4) reducing the required R.P. value from [REDACTED] (b)(4) [REDACTED] (b)(4).

Dr. George P. Shibley  
 7 March 1986  
 Page 3

As such we propose acceptance of the claims detailed in Table 3.

Table 3

<u>Product Code</u>	<u>Trade Name</u>	<u>Species Protected</u>	<u>Duration of Protection</u>	<u>MI Dose</u>	<u>Relative Potency at Release</u>
(b)(4)		Canine	1 year	(b)(4)	(b)(4)
		Feline	1 year		
(b)(4)		Canine	3 years	(b)(4)	(b)(4)
		Feline	3 years		
		Ovine	3 years		
		Bovine	1 year		
		Equine	1 year		

\*Change from currently approved Production Outlines.

We request immediate approval of the attached Production Outline page changes for Product Code (b)(4) on Product Code (b)(4) change submissions that provide for increase of the dose recommendation for sheep from (b)(4)

Deeply appreciating your consideration, I remain:

Sincerely,

(b)(6)

Attachments

MAR 12 1986

# LABORATORY TECHNIQUES IN RABIES

THIRD EDITION

EDITED BY

MARTIN M. KAPLAN

World Health Organization,  
Geneva, Switzerland

HILARY KOPROWSKI

Director, The Wistar Institute,  
Philadelphia, Pa., USA

*NIH 1st 3 → NIH 2nd 6*



WORLD HEALTH ORGANIZATION

GENEVA

1973

$$RP_{SD} = AV \times \frac{SD_{TV}}{SD_{RV}}$$

where SD = single dose (ml) for man  
 TV = test vaccine  
 RV = reference vaccine

It is presumed that 2 ml of the reference vaccine adjusted to 5% brain tissue suspension, represents a single dose for man. Suppose a single dose of the vaccine under test is given as 4 ml, then

$$RP_{SD} = 3.1 \times \frac{4}{2} = 6.2$$

## 2. Volumetric method

For the more recently developed vaccines prepared from virus suspensions whose nature, concentration, and purity are different from those of classical nervous tissue vaccines the gravimetric NIH-test is inapplicable. This is true of the suckling mouse brain vaccine containing only 1% of nervous tissue (see chapter 23), duck-embryo vaccine prepared from a 33% tissue suspension (see chapter 27), and tissue culture vaccines of which the concentration of the original virus harvest may be about 90% (see chapter 28). For these vaccines the volumetric test should be used. This compares the 50% endpoint *dilution* (highest vaccine dilution protecting 50% of mice) of the vaccine under test with that of the International Reference Vaccine (or equivalent national reference vaccine).

### Example :

Unlike the gravimetric method, this method does not require the International Reference Vaccine to be adjusted to the same concentration of virus harvest as the test vaccine. To determine 50% endpoint dilutions the reference vaccine is reconstituted as instructed and serial dilutions are made starting from this suspension, which is considered to contain 10% of brain tissue and of which 1 ml represents a single dose for man. At the same time, parallel serial dilutions of the vaccine under test are made, starting from the prescribed dilution for administration to man.

The relative potency (RP) of the vaccine under test is determined by the formula :

$$RP = \frac{\text{reciprocal of 50\% endpoint dilution of TV}}{\text{reciprocal of 50\% endpoint dilution of RV}} \times \frac{SD_{TV}}{SD_{RV}}$$

Suppose the following 50% endpoint dilutions were obtained :

$10^{-1.18}$  for the reference vaccine (reciprocal =  $10^{1.18}$ )

$10^{-1.67}$  for the test vaccine (reciprocal =  $10^{1.67}$ )

Furthermore assume that a single dose for man of the vaccine under test is given as 2 ml and that 1 ml of the reference vaccine represents a single dose for man, then

$$RP = \frac{10^{1.67}}{10^{1.18}} \times \frac{2}{1} = 10^{0.49} \times 2 = \text{antilog } 0.49 \times 2 = 3.1 \times 2 = 6.2$$

If a national reference vaccine is used that differs in potency from the reconstituted International Reference Vaccine, the result obtained with the above formula has to be multiplied by the RP of the national reference vaccine in terms of the International Reference Vaccine.

#### Minimum Potency Requirements

Where the gravimetric method is used the antigenic value (AV) must be at least 0.3. Manufacturers should be prohibited from increasing the brain tissue concentration and/or the volume of a single vaccine dose should the antigenic value of the brain tissue in the vaccine under test prove to be less than 0.3. A single dose for man must contain at least 100 mg of brain tissue (see above).

Where the volumetric test is used, the RP should be at least 0.3. If the RP of a vaccine under test exceeds this figure the final bulk should *not* be diluted nor should the volume of a single immunizing dose for man be reduced below the figure recommended by the producer at the time of the test.<sup>1</sup>

<sup>1</sup> Sixth report of the WHO Expert Committee on Rabies (*Wld Hlth Org. techn. Rep. Ser.*, 1973, No. 523). These requirements are under consideration by the WHO Expert Committee on Biological Standardization.



MAR 12 1986

**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

January 13, 1984

MERIEUX RABIES PROJECT

IMMUNOGENICITY TEST ON HORSES

- 16 month challenge -

VACCINE

(b)(4) similar to (b)(4)  
details in Appendix I).

VACCINATION

Twenty-six adult horses were vaccinated (b)(4)  
on (b)(4) against Rabies at Alba, a property belonging to  
(b)(4) and located 150 miles South of Lyon (France).

OBSERVATIONS (until challenge):

All horses remain in good health. (b)(4) at the end of  
1982 before decision of challenge.

TESTING

1. Serology:

a) Blood sampling:

Before vaccination, (b)(4) months after vaccination all  
horses were bled in the jugular vein for serum sampling.

b) Results:

Antibody titers expressed in log (b)(4)

Before vaccination, all horses are (b)(4) as well as  
the five controls before challenge.

(b)(4) on vaccinated horses is satisfactory.

**2. Challenge:**

. Location: In animal facilities of (b)(4) (France)  
on October 14, 1983.

. Challenge strain: (b)(4) virus strain, suspension of dog  
(b)(4)

Prepared in Lyon from suspension of dog salivary gland obtained from  
CDC, Atlanta.

**. Animals:**

- (b)(4)
- Seven vaccinated horses selected for challenge by (b)(6)  
on (b)(4) horses transferred from Alba to  
Lyon.

**. Method:**

Preparation of a dilution (b)(4)

- 1) (b)(4)
- 2) (b)(4)

This dilution corresponding to (b)(4) been selected  
from results of a preliminary challenge started on 6/2/83, (Appendix II).

Inoculation of challenge strain in horses: under contention, (b)(4)  
(b)(4) at two sites in the masseter muscles of each  
horse (b)(4)

(b)(4) challenge virus on mice.

After the completion of the horse challenge, (b)(4)  
the viral suspension are prepared. (b)(4)  
per dilution by (b)(4)

**. Observations:**

Daily observation for 28 days on mice and 48 on horses.

**. Results:**

a) (b)(4)  
Titer of challenge virus: (b)(4)

Each horse receives (b)(4) considering this (b)(4)  
(b)(4) considering the average titer of this virus.

**b) horses:**

(b)(4) challenge, all vaccinated horses are healthy and  
4 out of 5 control died between Dec 19 and Dec 22, 1983.

. Conclusion [REDACTED] challenge:

With [REDACTED] the test is valid and reveals that Rabisin batch [REDACTED] did protect the seven horses vis-a-vis the inoculation of a NYC challenge strain 16 months after vaccination.

[REDACTED]  
(b)(6)

Table 1

(b)(4)

ON HORSES

Rabies Ab in log 10

(b)(4)

Horses Ref.	No.	Time after vaccination (in months)			
		2 m.	7 m.	13 m.	16 m.
3721	1	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3723*	2	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3724	3	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3725*	4	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3726	5	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3727*	6	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3728	7	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3729	8	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3730	9	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3731*	10	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3733*	11	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3735	12	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3736	13	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3738	14	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3739	15	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3797	16	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3722	17	(b)(4)	(b)(4)	(b)(4)	(b)(4)
	18	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3799	19	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3801	20	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3802*	21	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3803	22	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3804	23	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3805	24	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3806	25	(b)(4)	(b)(4)	(b)(4)	(b)(4)
3807	26	(b)(4)	(b)(4)	(b)(4)	(b)(4)

\* Challenged horses 16 months after vaccination.

The numbers in parenthesis at 16 months express the antibody value using the SN mouse test.



Appendix I: Batch 10G191 NIH

Vaccine	Dilutions					PS
	1/5	1/25	1/125	1/615	1/3125	
(b)(4)						
(b)(4)						

\* No. dead mice/No. inoc. mice

Challenge dose: (b)(4)

PNR009 is a sub.reference vaccine distributed on (b)(4) by the Centre d'Etudes Nationales sur la Rage (C.E.N.R.), Nancy, a department of the French Ministry of Agriculture specialized on Rabies. A document published by the C.E.N.R. on (b)(4) is 1.6 times more potent than reference vaccine NIH81.

Therefore (b)(4)

RABIES VACCINE, KILLED VIRUS  
HAMSTER CELL LINE ORIGIN

MAR 12 1986

C. Immunogenicity tests on sheep

EXPERIMENT # 1

Vaccination:

(b)(4)

Challenge:

(b)(4)

Results:

(b)(4)

EXPERIMENT # 2

Vaccination:

Combined  
negative

(b)(4)

Challenge:

42-44 months after vaccination, challenge of (b)(4) and of  
15 controls with NYC strain (b)(4)

Results:

See table 3  
(b)(4) resisted the challenge whereas (b)(4)

EXPERIMENT # 3

Vaccination:

Group a):

(b)(4)

Group b)

(b)(4)

Challenge: 36 months after vaccination, challenge of

(b)(4)  
(b)(4)

Results:

See table 4 and fig.1.

(b)(4)

Table 1. Potency test results of rabies vaccine serials used for the immunogenicity tests.

Vaccine #	Date of Test	Tested vaccine				Protected/Challenged				CVS challenge virus (Dead/Inoc.)				Inoc. dilution	Mouse potency (tested virus)	Mouse potency (sub-ref. vaccine)	NIH	(Sub-ref. vaccine) / Ref. vacc.
		1/5	1/25	1/125	1/625	No.	1/5	1/25	1/125	1/625	-4.6	-5.6	-6.6					
(b)(4)																		

- 1 dose AH vaccine: 1 ml
- 1 dose AH+S vaccine: 2 ml
- 1 dose (Atrab+FMD) vaccine: 5 ml

Table 2. Persistence of immunity in sheep vaccinated SQ with (AH+S) vaccine.

Blood Sampling #	Time (months)						Challenge			
	1	2	3	6	9	12	16	24	before	after
(b)(4)										

- 1) Sheep died accidentally before challenge
- 2) 38-44 months: challenge with 2700-9000 MLD<sub>50</sub>

Titers are expressed in serum dilution log<sub>10</sub>

Challenge:  
Vaccinated: 19/19 protected  
Controls: 17/20 died

Table 3. Persistence of immunity in sheep  
vaccinated SQ with combined (AH-rabies+FMD) vaccine.

Blood Sampling #	Time (months)							Challenge		
	1	2	3	6	9	12	16	24	before	after
1	(b)(4)									
2										

- 1) Sheep died accidentally before challenge
  - 2) 42-44 months: challenge with 2700/5300 MLD<sub>50</sub>
- Titers are expressed in serum dilution log<sub>10</sub>

Challenge:

Vaccinated: 18/18 protected  
Controls: 12/15 died

Table 4. Persistence of immunity in sheep vaccinated SQ with (AH+S) vaccine (a) or with AH vaccine (b).

Blood Sampling #	Time (months)				Challenge	
	1	3	6	12	before	after
(b)(4)						

(1) Sheep died before challenge  
 (2) (3) 36 months: Challenge with 5300 MLD<sub>50</sub>

Titers are expressed in serum dilution log<sub>10</sub>

Challenge:

Vaccinated 21/21 protected

Controls: 8/8 died

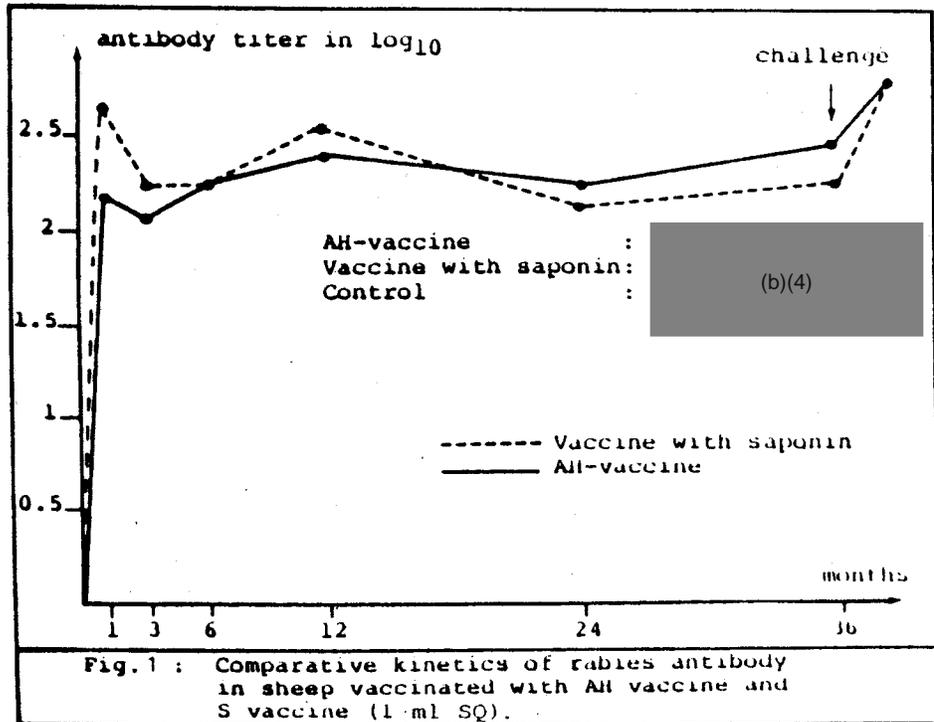


Fig. 1 : Comparative kinetics of rabies antibody in sheep vaccinated with AH vaccine and S vaccine (1 ml SQ).



MAR 12 1986

**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

23 October 1985

## RABIES

3-YEAR (b)(4)  
 IN DOGS

Summary  
 see p. 4

\_\_\_\_\_  
 PROTOCOL/RESULTS

1.0 PROTOCOL1.1 Vaccine:

(b)(4) was used to immunize the dogs in this trial. Using 16-18 g mice, the NIH value was found to be (b)(4) to reach a (b)(4) prior to administration. (b)(4) satisfactory for purity, safety and potency per 9 CFR 113.129.

1.2 Dogs/Vaccination:

All were received from a beagle colony at (b)(4) Inc. These dogs were subcutaneously vaccinated with (b)(4) of vaccine on (b)(4) of age.

1.3 Size/Sex/Age of Challenge Groups:

27 vaccinated (16 male, 11 female) and 28 unvaccinated (12 male, 16 female) controls were challenged. The age of the non-vaccinated dogs added to the study later were (b)(4) at challenge as compared to (b)(4) for the vaccinates and other (b)(4)

1.4 Challenge Virus:

The challenge virus was a canine salivary gland suspension of (b)(4). The challenge virus was (b)(4) (b)(4). The challenge virus was titered in mice to calculate the actual (b)(4)

(b)(4)

3-Year (b)(4)  
Protocol/Results  
Page 2

### 1.5 Method/Date of Challenge:

The challenge virus was given bilaterally into the temporal muscles, (b)(4) per dog. A new needle was used for each injection. A separate syringe was used for each dog. All dogs were challenged on (b)(4)

The dogs were challenged in alternating fashion with vaccinates and controls; i.e., the vaccinates and controls were randomly mixed together in four rooms prior and then removed singly for challenge, etc.

### 1.6 Serology:

Serum virus neutralization titers were conducted on serum samples of all dogs before vaccination and in all vaccinated dogs at months (b)(4) and in all dogs surviving challenge; i.e., 3-months post challenge.

Rabies neutralizing antibody levels were determined by the (b)(4) (Smith, J.S., P.A. Yager, and G.M. Baer. 1973) A rapid reproducible test for determining rabies neutralizing antibody. Bull. Wld. Hlth. Org. 48:535-541.) The test was adapted so that (b)(4) The cell line used was (b)(4) rabies virus strain was (b)(4)

### 1.7 Holding Period Following Challenge:

All challenged dogs were kept until death or for (b)(4) months following challenge, whichever came first. Completion of the test was (b)(4)

### 1.8 Confirmatory Procedures:

Brain tissues were collected from all dogs and mice dying following challenge. Impression smears of brain tissue were observed by (b)(4) to confirm rabies.

### 1.9 Incidental Deaths:

One dog (b)(4) died of a reported (b)(4) on (b)(4) post-vaccination) at the holding facilities. No other deaths or deletions are evident.

### 1.10 General:

The vaccinates as well as controls were each housed in separate enclosures during the post challenge period so as not to distort challenge results in the control dogs.

## 2.0 RESULTS

### 2.1 Challenge Virus Titer:

The median mouse (b)(4) when titered by the (b)(4) when titered by the intramuscular route (see Table 1).

The actual challenge dose given to each dog was (b)(4) (b)(4) The titer was conducted on the (b)(4) immediately following completion of the dog challenge.

### 2.2 Vaccination/Challenge Rabies Confirmation:

Twenty-eight of twenty-eight (28/28) rabies susceptible controls died between 11 and 17 days post challenge (see Table 2). Acetone-fixed impression smears from all 28 dogs were stained with a (b)(4)

(b)(4)  
 The specificity of the conjugate was confirmed by reacting it with the following control materials: impression smears prepared from normal dog brain (b)(4) brain impression smears from mice intracranially inoculated with challenge virus (b)(4) and brain impression smears from one of the susceptible dogs that died of rabies in the preliminary challenge evaluation (b)(4) Specific (b)(4) antigen was present in impression smears prepared from the brain of each of the dead (b)(4)

One of twenty-seven vaccinates (1/27) (b)(4) died on (b)(4) challenge. Rabies infection of the brain was confirmed by (b)(4)

### 2.3 Serology:

Serological data for each dog is reported for months (b)(4) (b)(4) post challenge (see Table 3). The mean titer of the protected vaccinates was (b)(4) a range of (b)(4) The one vaccinate that died (b)(4) The titers of this dog were low throughout the test for some unknown reason.

The mean (b)(4) titers were as follows all dogs:

Months post-vacc	(b)(4)
Mean titer	(b)(4)

As each of these bleeds/antibody determinations were conducted independent of each other, there will be some modest variation in results. This data does not seem to indicate a statistically significant increase in mean titer at either (b)(4) post-vaccination. What does appear evident is a very consistently high antibody response from one vaccination that persists at least for three years.

3.0 SUMMARY

The three year (b)(4) indogs was satisfactorily concluded with (b)(4) in dogs receiving (b)(4) of Merieux Rabies Vaccine, Killed Virus with an NIH value of (b)(4) should be noted that this vaccine antigen was approximately 1 year old when administered to dogs in this test.) All 28/28 unvaccinated controls (b)(4) challenge. Rabies virus presence was confirmed by (b)(4) brain in all dogs that died in the test.

Serological antibody titers were consistently high in all vaccinated throughout the 3 year post vaccination except for (b)(4) consistently declined over (b)(4) month period (b)(4) A majority of the vaccinates (17/26) responded to challenge with an increase in (b)(4) antibody.

The study demonstrated persistent protective rabies (b)(4) in dogs for three years (b)(4)

(b)(6)

Attachments:

- Table 1 - Titrations of Challenge (b)(4) Mice
- Table 2 - Response of Dogs (b)(4) with Virulent Rabies Virus
- Table 3 - Serological Data

**Table 1. Titration of Rabies Virus Challenge Inoculum**

**Rabies Titration**

**Date:** 5/24/85

**Route:** I.C.

**Number of Mice Alive**

**Dilution**

**Day Post Challenge**

(b)(4)

(b)(4)

2.0

**Median mouse**

**lethal dose =**

(b)(4)

(b)(4)

+

(b)(4)

(b)(4)

**Rabies Titration**

**Date:** 5/24/85

**Route:** I.M.

**Number of Mice Alive**

**Dilution**

(b)(4)

(b)(4)

1.1

**Median mouse**

**lethal dose =**

(b)(4)

(b)(4)

Table 2. (b)(4) Three Year Duration of (b)(4)  
 Responses of Dogs Intramuscularly Challenged (b)(4)  
 With Virulent Rabies Virus Inoculum.

Dog Number	Vaccination Status	Day Post Chal.		F. A. Test
		Onset of Illness	Death	
AAVCZ 634	Control			Pos.
CAHFM 3	Vaccinate			
HBVDI 4	Vaccinate			
HBXDG 1	Control			Pos.
HDHCX 314	Control			Pos.
HEKDA 4	Control			Pos.
HEL 13 1	Vaccinate			
HFZDH 4	Vaccinate			
HJMEL 1	Control			Pos.
HJP 3 2	Vaccinate			
HJPES 3	Vaccinate			
HJPES 6	Control			Pos.
HKREN 214	Control			Pos.
HMVFJ 1	Vaccinate			
HOACZ 414	Control			Pos.
HQJFK 1	Vaccinate			
HSJEZ 1	Control			Pos.
HSJFD 614	Control			Pos.
HSLFZ 714	Control			Pos.
HSNEZ 4	Vaccinate			
HTTFT 5	Vaccinate			
HUA 6 2	Control			Pos.
HUA 6 5	Vaccinate			
HUA 6 7	Vaccinate			
HUMEC 2	Vaccinate			
HUMEC 3	Vaccinate			
HUQEK 3	Vaccinate			
HUS 7 3	Control			Pos.
HVIEI 3	Vaccinate			
HVJFE 4	Control			Pos.
HWHFH 1	Vaccinate			
HWR 5 1	Vaccinate			

(b)(4)

Table 2 (Continued):

Dog Number	Vaccination Status	Day Post Chal.		F. A. Test
		Onset of Illness	Death	
HWR 5 2	Control			Pos.
HWR 5 4	Vaccinate			
HWR 5 6	Vaccinate			
HYGFO 6	Control			Pos.
HZZFD 4	Control			Pos.
HZZFD 8	Control			Pos.
RCB 5 5	Vaccinate			
RJDCH 634	Control			Pos.
RJYBX 214	Control			Pos.
RKWDG 314	Control			Pos.
RPNEF 514	Control			Pos.
RPNEF 614	Control			Pos.
RQICE 114	Control			Pos.
RRQCI 314	Control			Pos.
RSHCK 114	Control			Pos.
RWJES 314	Control			Pos.
TADFX 3	Vaccinate			
TBDEA 1	Control			Pos.
TBI 24 2	Vaccinate			Pos.
TBREN 2	Vaccinate			
TCXFW 3	Vaccinate			
TCXFW 6	Vaccinate			
TDJEA 2	Vaccinate			

(b)(4)

TABLE 3

AB<sup>tm</sup> - Canine three-year duration of  
Antibody Titers

(b)(4)

(b)(4)

Dog Number	Months Post Vaccination									Months Post Challenge
	0	1	3	6	9	12	17	24	37	3
1 TDJEA #2										
2 HEL13 #1										
3 HVIEI #3										
8 HWR-5 #6										
10 CAHFH #3										
12 HFZDH #4										
14 TBI24 #2										
26 HJP 3 #2										
27 TBREN #2										
28 HWR 5 #4										
41 HBVDI #4										
42 HUQEK #3										
43 HMOVJF #1										
44 HQJFK #1										
45 TCXFW #3										
46 HUA 6 #7										
47 HTTFT #5										
48 HSNEZ #4										
49 HUA 6 #5										
50 HJPES #3										
51 TADFX #3										
52 HWR 5 #1										
53 HUMEC #2										
54 HWBFH #1										
55 HUMEC #3										
57 TCXFW #6										
17 RCB 5 #5										
ALL CONTROLS										

(b)(4)

MAR 12 1986



**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

February 9, 1983

Dr. G.P. Shibley  
 Veterinary Services  
 USDA-APHIS  
 Federal Building  
 Hyattsville, Maryland 20782

[Redacted] (b)(4)

on Cattle

Dear Dr. Shibley:

I would like you to authorize Merieux Laboratories to have [Redacted] (b)(4) vaccine recommended for use not only in dogs and cats but also in horses, cattle and sheep.

This request for use in other species is supported by data obtained at the Institut Merieux, Veterinary Department, Lyon, France, during the past twelve years (see enclosed report).

As mentioned in this report, more than [Redacted] (b)(4) have been used for horses and 20,000,000 for cattle and sheep. No failure of vaccination or abnormal reaction has been reported.

The data with the saponine adjuvanted vaccine have been included because we believe that, on cattle and sheep, [Redacted] (b)(4) has the same effect, that is to say, that the two vaccines are comparable (see tables 19 and 20).

Sincerely,

[Redacted] (b)(6)

Dr. Shibley

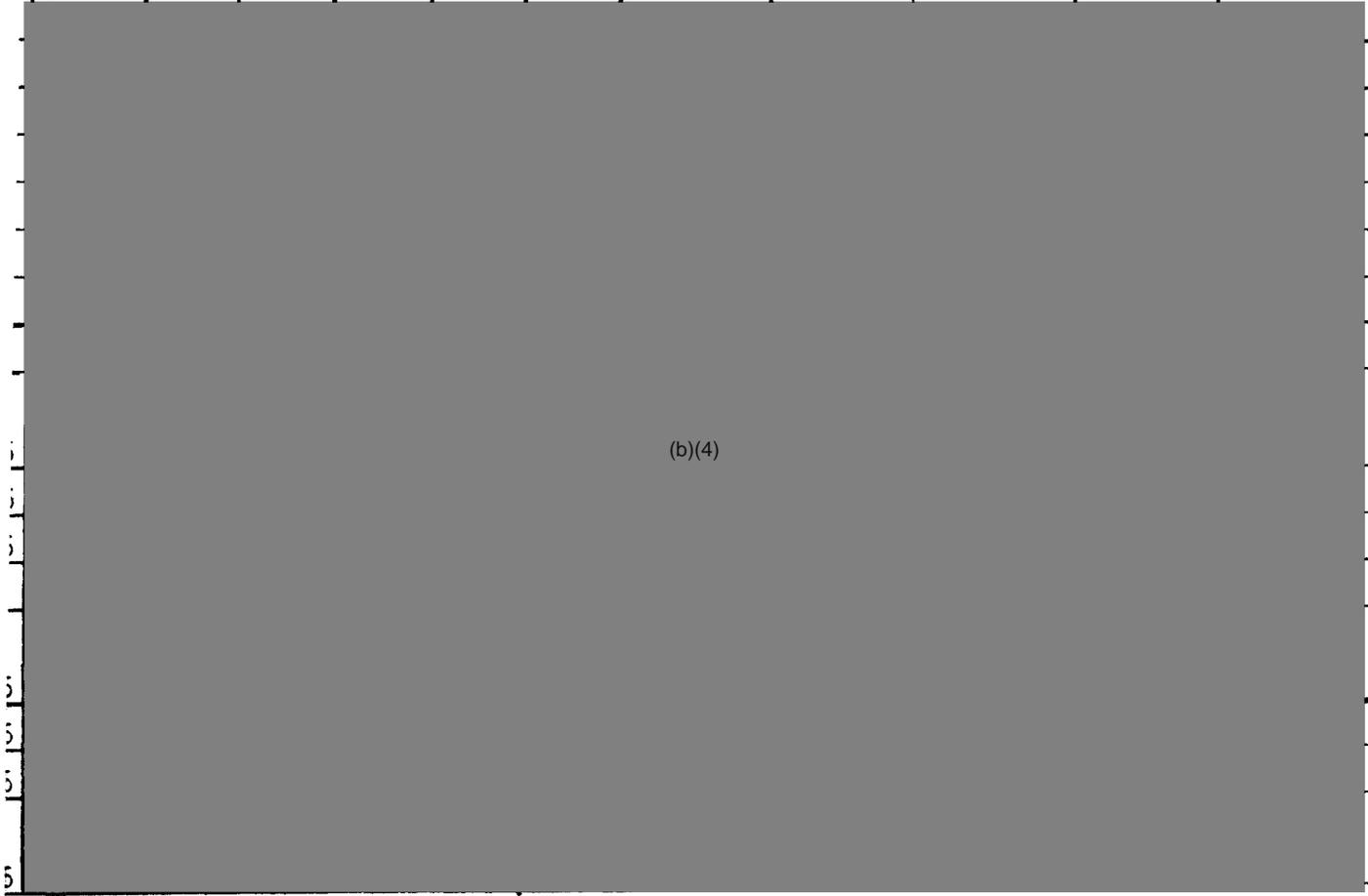
4 March 1986

Note: See Specifically Exp. # 6  
 on p.12 and p.17 (Table 10)  
 and p.18 (Figure 5)

DA.

abies vaccine serials used for the immunogenicity tests.

Vaccine		CVS Challenge Virus				Inoc. dilution	Mouse potency (tested virus)	Mouse potency (sub-ref. vaccine)	NIH/ml	(Sub-vaccine) Ref. Vacc.
		(Dead/Inoc.)								
1/125	1/625	-4.6	-5.6	-6.6	-7.6					



(b)(4)

RABIES VACCINE, KILLED VIRUS  
HAMSTER CELL LINE ORIGIN

E. (b)(4) on cattle

EXPERIMENT # 1 (1)

Vaccination:

- AH + S vaccine inoculated SQ to 16 (b)(4) seronegative cattle

- A booster was carried out sixteen months after vaccination on (b)(4)

Challenge:

None.

Results:

Kinetics of rabies antibody : See table 6.

EXPERIMENT # 2 (1)

Vaccination:

- Combined (AH-rabies + FMD) vaccine inoculated SQ to (b)(4)

- A booster was carried out sixteen months after vaccination on (b)(4)

Challenge:

None.

Results:

Kinetics of rabies antibody: See table 7.

EXPERIMENT # 3 (2)

Vaccination:

- (b)(4)  
(b)(4)

- A booster was carried out 12 months after vaccination on (b)(4)

Challenge:

None.

Results:

(b)(4) antibodies: See fig. 3 ( non boosted cattle only) and table 8.

EXPERIMENT # 4 (2)

Vaccination:

- [redacted] (b)(4)

- A booster was carried out 12 months after vaccination on [redacted] (b)(4)

Challenge:

None.

Results:

Kinetics of rabies antibody: See fig. 3 (non boosted cattle only) and table 9.

Note:

Experiment # 3 and # 4 were carried out in parallel on the same group of cattle.

EXPERIMENT # 5 (3)

This experiment was sponsored by the United Nations Development Program U.N.D.P.), Research Program on paralytic rabies [redacted] (b)(4)

Vaccination:

AH + S vaccine inoculated to [redacted] (b)(4) (ten with [redacted] (b)(4) [redacted] (b)(4) from same group kept as controls.

Challenge:

- Among the 30 animals, [redacted] (b)(4) 3 year observation period from troubles not connected with rabies or vaccination.

- At the date of challenge (36 months after vaccination, 26 animals were remaining:

- Vacc. [redacted] (b)(4)
- Vacc. [redacted] (b)(4)
- Controls: [redacted] (b)(4)

Each of them was challenged with Bat origin rabies virus, [redacted] (b)(4) in the masseter muscle.

Results:

- Antibody kinetics and challenge results: See fig. 4.

- 9/9 vaccinated with [redacted] (b)(4) the challenge, whereas 8/8 controls died from rabies [redacted] (b)(4) after challenge.

Vaccination:

AH va (b)(4) ulated SQ to 10 rabies seronegative  
cattl (b)(4)

Challenge:

(b)(4) vaccination, challenge of the 10 vaccinated  
and 5 controls, with strain BAT-DR19 inoculated in the  
masseter muscle.

Results:

(b)(4) were protected: 5/5 controls died.  
- Antibody kinetics: See table 10 and fig. 5.

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- (1) Institut Merieux, Veterinary Dept., Lyon, France. (b)(4)
- (2) Institut Merieux, Veterinary Dept., Lyon, France. (b)(4)
- (3) Hernandez Baumgarten E., et al.: "Evaluacion de una vacuna comercial antirrabica inactivada para bovinos, producida in cultivo de tejidos (Alurabiffa). Duracion de inmunidad con desafio a tres anos." Technica Pecuaria en Mexico, 1976, 30, p. 57-63.
- (4) Petermann H.G. et al., "La vaccination antirabique des carnivores et des herbivores avec un vaccin inactive produit sur culture de tissus." Bull. Soc. Sci. Vet. Med. Comp. Lyon, 1971, 73, 123-141.

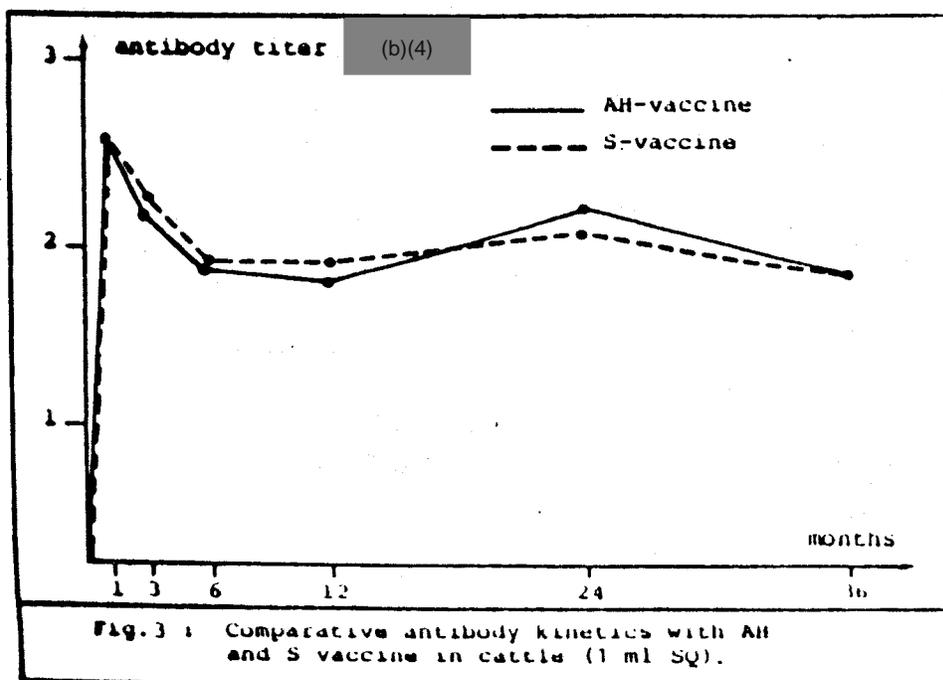


Table 6. Kinetics of rabies antibody in cattle vaccinated SQ with AH+S vaccine.

Bovine #	Time after vaccination (in months)				
	1	3	6	16	28
21	(b)(4)				
22					
23					
24					
25					
26					
27					
28					
612					
601					
607*					
606*					
620*					
603*					
623*					
624*					
Mean:					

\* Booster 16 months after vaccination.

Table 7. Kinetics of rabies antibody in cattle vaccinated SQ with combined (AH-rabies+FMD) vaccine.

Bovine #	Time after vaccination (in months)					
	1	3	6	16	18	40
1	(b)(4)					
2						
3						
4						
5						
6						
7						
8						
9						
10						
11*						
12*						
13*						
14*						
15*						
16*						
17*						
18*						
Mean						

\* Booster 16 months after vaccination.

Table 8. Kinetics of rabies antibody in cattle vaccinated SQ with AH + S vaccine.

Bovine #	Time after vaccination (in months)					
	1	3	6	12	24	36
1040	(b)(4)					
1851						
1852						
1854						
1865						
1867						
1868						
1700 *						
1844 *						
1856 *						
1859 *						
1860 *						
Mean						

\* Booster 12 months after vaccination.

**Table 9. Kinetics of rabies antibody in cattle vaccinated SQ with AH vaccine.**

Bovine #	Time after vaccination (in months)					
	1	3	6	12	24	36
1619	(b)(4)					
1843						
1845						
1848						
1857						
1559						
1699*						
1842*						
1849*						
1855*						
1857*						
1861*						
1863*						
Mean:						

\* Booster 12 months after vaccination.

Table 10. Kinetics of rabies antibody in cattle vaccinated with AH vaccine (2 ml, SQ)

Cattle #	Time after vaccination (in months)					
	1	2	4	8	10	16
172	(b)(4)					
195						
199						
201*						
202						
205						
206						
207						
209						
214						
Mean						

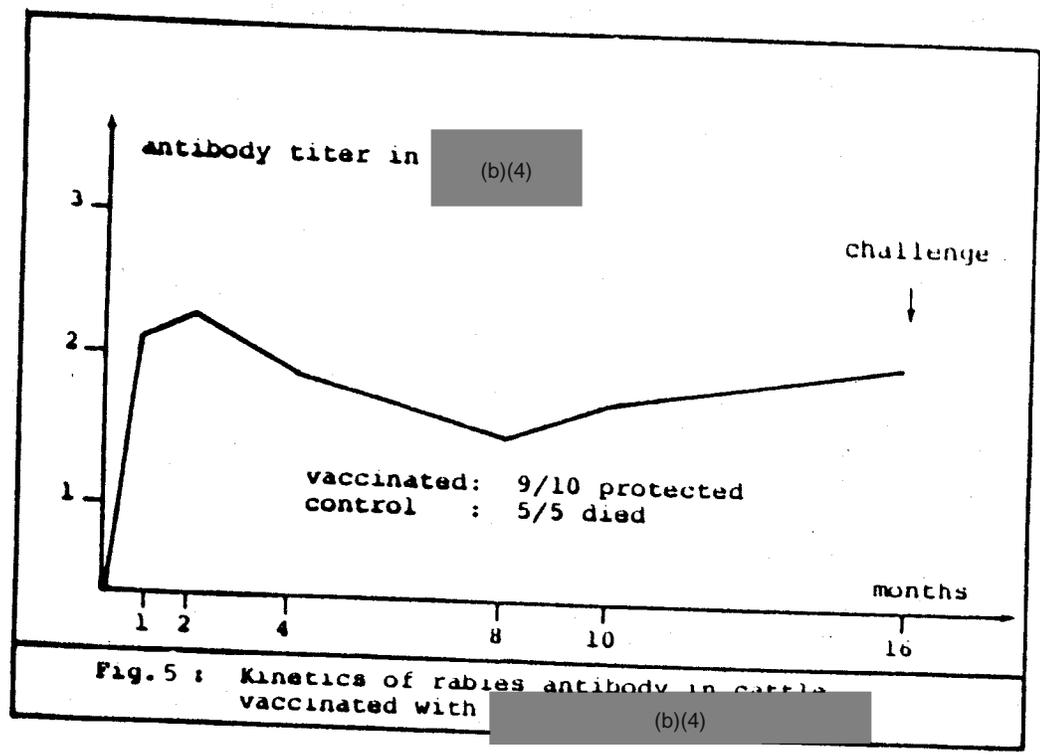
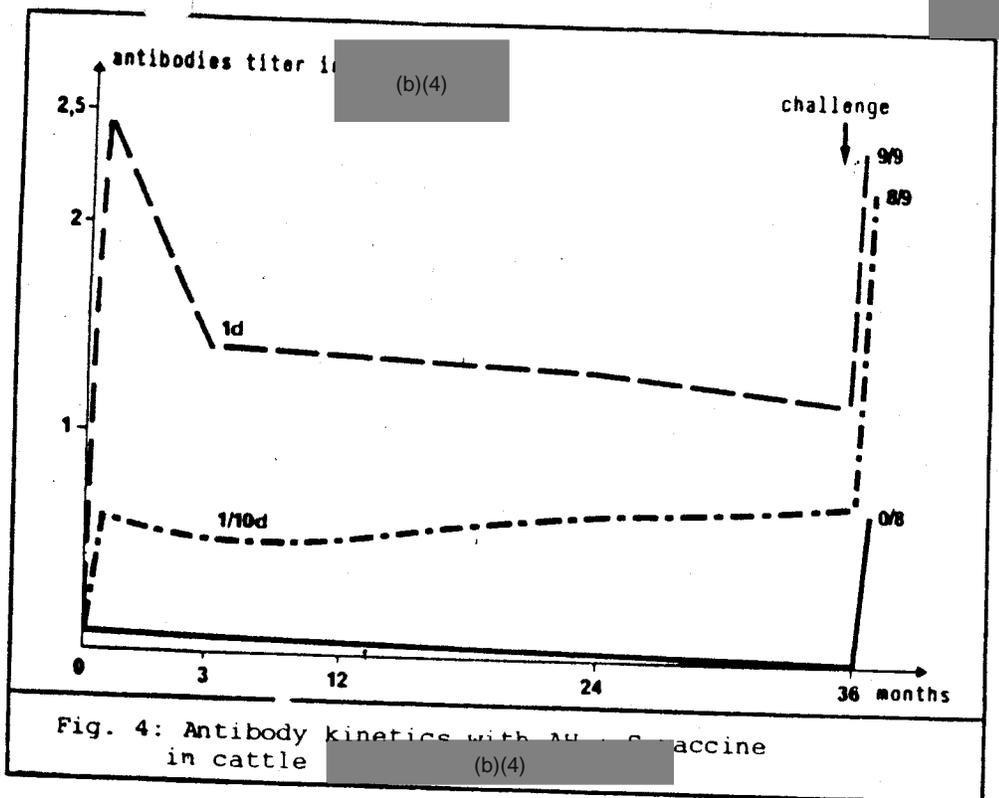
Challenge at 16 months

Challenge:

Vaccinated: 9/10 protected

Controls: 5/5 died

\* # 201 did not resist the challenge even with an antibody titer of 2.12. One possible hypothesis is that challenge virus has been inoculated in peri-nerval area.





MAR 12 1986

**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

1 March 1986

RABIES

3-YEAR FELINE (b)(4)

PROTOCOL/RESULTS

1.0 PROTOCOL

1.1 Vaccine

Vaccine serial (b)(4) was used to immunize the cats in this trial. Using 16-18g mice, the NIH value was found to be (b)(4) to reach a theoretical (b)(4) administration. (b)(4) satisfactory for purity, safety and potency per 9 CFR 113.129.

1.2 Cats/Vaccination

All were received from Liberty Labs at Liberty Corner, NJ. The cats in the Duration of Immunity Trial were subcutaneously vaccinated with (b)(4) when (b)(4)

1.3 Size/Sex of Challenge Groups

Twenty-nine vaccinated female cats and a total of 26 unvaccinated female controls were challenged.

1.4 Preliminary Challenge

A preliminary challenge in (b)(4) years of age, and (b)(4) of age from Merieux Laboratories) was initiated on (b)(4) the (b)(4) facilities in (b)(4)

1.5 Challenge Virus

The challenge virus was a canine salivary gland suspension of (b)(4). The challenge virus was diluted in order to inject approximately (b)(4). The procedure for diluting the salivary gland suspension was as follows: (b)(4) were thawed, pooled and (b)(4) etal calf

(b)(4)

Rabies 3-Year Feline  
Protocol/Results  
Page 2

(b)(4)

serum (b)(4) to make a working  
stock virus pool of 1/15 dilution.

### 1.6 Mouse Titrations

The infectivity of each virus inoculum was determined by titration in mice. After all the cats had been inoculated, a portion of the working stock virus pool was diluted serially for titration in mice.

#### 1.6.1 Preliminary Challenge (3 October 1985):

Groups of ten mice were inoculated intramuscularly with (b)(4) and (b)(4)

Twenty mice were inoculated intracranially with (b)(4) virus dilution; ten mice per group were inoculated with (b)(4)

#### 1.6.2 Duration of (b)(4) (22 November 1985):

Groups of ten mice were inoculated intramuscularly with (b)(4) dilutions of virus: (b)(4)

Twenty mice per group were inoculated intracranially with (b)(4) ten mice per group were inoculated with dilutions of (b)(4)

Three weeks after inoculation, the median mouse lethal dose of the challenge inoculum was calculated for each route of administration. Endpoints were calculated by the Spearman-Kärber method.

### 1.7 Method/Date/Place of Challenge

Each cat was inoculated intramuscularly with (b)(4) (b)(4) The inoculum was administered bilaterally into the dorsal muscles of the neck. (b)(4) volume of the challenge virus inoculum was injected at each site. A new needle was used for each injection.

In the Duration of (b)(4) alternating inoculation of the vaccinated and susceptible control cats was practiced. The cats were caged individually throughout

(b)(4)

the trials.

All cats in the Duration of (b)(4) were challenged  
(b)(4) facilities in (b)(4)  
(b)(4)

### 1.8 Serology

Serum virus neutralization titers were conducted on serum samples of all cats before vaccination and in all vaccinates at months (b)(4) months post-vaccination. (The pre-challenge serum samples were taken just prior to challenge at Pitman-Moore.)

Rabies neutralizing antibody levels were determined by the (b)(4). (Smith, J.S., P.A. Yager, and G.M. Baer. 1973. A (b)(4) test for determining rabies (b)(4) Bull. Wld. Hlth. Org. 48:535-541.) The test was adapted so that microtitration equipment could be used. The cell line used was (b)(4) the rabies virus strain was (b)(4)

### 1.9 Confirmatory Procedures

(b)(4) Acetone-fixed impression smears were prepared from the brain of each cat that died in the Duration of (b)(4). The slides were stained with a (b)(4) for (b)(4) the presence of rabies virus antigens in neurons.

### 1.10 Incidental Deaths

Cat # 82PE1 died on (b)(4) due to causes unrelated to vaccination. Cat # 82NR3 was accidentally crushed by the animal caretaker and died on (b)(4) was cannibalized by the other cats because of a prolapsed rectum and died on (b)(4). No other deaths or deletions are evident during the pre-challenge period.

No incidental deaths occurred during the post-challenge period in any vaccinated cats.

### 1.11 General

The vaccinates as well as controls were housed in separate enclosures during the post-challenge period so as not to distort challenge results in the control animals.

## 2.0 RESULTS

### 2.1 Challenge Virus Titer

2.1.1 The mouse (b)(4) (b)(4) are included in Table 1. The infectivity of the (b)(4) administered to the cats was estimated to be (b)(4) (b)(4) rabies virus per ml.

2.1.2 The inoculum prepared for the Duration of (b)(4) was estimated to contain (b)(4) (b)(4) of rabies virus per dose (Table 2).

### 2.2 Cat Challenge Trials

In the Preliminary Challenge Trial, 9 of 10 susceptible cats died with neurologic signs of rabies after being inoculated with the (b)(4) rabies virus. Clinical signs of rabies appeared in affected cats between the (b)(4) after challenge. The cats died from (b)(4) (b)(4) after inoculation.

In the Duration of (b)(4) susceptible control cats developed signs of rabies and died. The onset of clinical signs ranged between (b)(4) (b)(4) days) after inoculation. The cats usually died (b)(4) after the onset of clinical rabies. The deaths occurred from (b)(4) after inoculation. Impression smears of brain tissue taken from each of the 12 cats have been demonstrated to contain rabies viral antigens by (b)(4)

(b)(4) cats inoculated with (b)(4) of the (b)(4) rabies virus - infected canine salivary gland developed clinical rabies and died.

All 29 of the vaccinated cats remained healthy, for 90 days post rabies virus challenge.

### 2.3 Serology

Serological data for each cat is reported for month (b)(4) (b)(4) (see Table 3). The mean titer of the vaccinates at 36 months post-vaccination was (b)(4) range of (b)(4)

The mean (b)(4) titers were as follows for all cats:

Months post-vacc	(b)(4)
Mean titer log <sub>10</sub>	(b)(4)
GMT (reciprocals)	(b)(4)

3.0 SUMMARY

The three year (b)(4) in cats was satisfactorily concluded with 100% protection (29/29) in cats receiving (b)(4) of Merieux Rabies Vaccine, Killed Virus with an (b)(4). (b)(4) should be noted that this vaccine antigen was approximately 1-1/2 years old when administered to cats in this test.) Twenty-one (b)(4) unvaccinated control cats inoculated with the (b)(4) rabies virus developed clinical rabies and died. Rabies virus presence was confirmed by (b)(4) brain in all cats that died in the test.

Serological antibody titers were consistently high in all vaccinated cats throughout the three year post vaccination period.

The study demonstrated persistent protective rabies (b)(4) in cats for three years post vaccination.

(b)(6)

(b)(6)

Attachments:

- Table 1: Preliminary Cat Challenge Trial
- Table 2: Duration of (b)(4)
- Table 3: (b)(4) Feline three-year Duration of (b)(4) Serum Neutralization Antibody Titers

**Table 1: Preliminary Cat Challenge Trial**

Cat Number	Group	Day Post Challenge	
		Appearance of Clinical Signs	Death
R - 132	Preliminary Challenge of Susceptible Cats	(b)(4)	(b)(4)
R - 133			
R - 141			
R - 142			
R - 143			
R - 153			
81 GG1			
81 GG15			
82 001			
82 003			

Mean:

**Rabies virus titrations:**

**Date:** October 3, 1985      **Route:** Intracranial

Number of Mice Alive

Dilution	Day Post Challenge																		
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	(b)(4)																		

Median mouse lethal dose =

(b)(4)

2.4

(b)(4)

Table 1 (Continued):

Route: Intramuscular

Day Post Challenge

Dilution	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	P
	[REDACTED]																			

3.4

Median mouse lethal dose = [REDACTED] (b)(4)

[REDACTED] (b)(4)

(Failure of the lowest dilution of the Spearman-Kärber test) [REDACTED] (b)(4) by

Table 2: Duration of Immunity Trial

Cat Number	Group	Day Post Challenge		FA Results		
		Appearance of Clinical Signs	Death			
82 OE 3	C	(b)(4)		+		
82 OF 2	C			+		
82 OJ 2	C			+		
82 OK 4	C			+		
82 OK 5	C					
82 OP 3	C			+		
82 OP 5	C			+		
82 OT 4	C					
82 OT 5	C					
82 OW 1	C					
82 OY 2	C					
82 OY 3	C					
82 PB 1	C					
82 PB 2	C					
82 PD 3	C					
82 PM 3	C					
				Mean:		
82 MC 3	V			Asymptomatic	Survived	
82 MF 3	V			Asymptomatic	Survived	
82 MF 4	V			Asymptomatic	Survived	
82 MU 1	V	Asymptomatic	Survived			
82 MU 2	V	Asymptomatic	Survived			
82 MU 3	V	Asymptomatic	Survived			
82 MV 3	V	Asymptomatic	Survived			
82 MW 2	V	Asymptomatic	Survived			
82 MX 3	V	Asymptomatic	Survived			
82 MY 4	V	Asymptomatic	Survived			
82 MZ 1	V	Asymptomatic	Survived			
82 MZ 2	V	Asymptomatic	Survived			
82 NA 4	V	Asymptomatic	Survived			
82 NB 2	V	Asymptomatic	Survived			
82 ND 5	V	Asymptomatic	Survived			
82 NE 3	V	Asymptomatic	Survived			
82 NF 5	V	Asymptomatic	Survived			
82 NH 1	V	Asymptomatic	Survived			
82 NH 2	V	Asymptomatic	Survived			
82 NI 5	V	Asymptomatic	Survived			
82 NN 5	V	Asymptomatic	Survived			
82 NO 2	V	Asymptomatic	Survived			
82 NR 1	V	Asymptomatic	Survived			
82 NT 3	V	Asymptomatic	Survived			
82 NU 4	V	Asymptomatic	Survived			
82 NY 3	V	Asymptomatic	Survived			
82 NY 4	V	Asymptomatic	Survived			
82 OA 2	V	Asymptomatic	Survived			
82 OE 2	V	Asymptomatic	Survived			

Table 2 (Continued)

Rabies virus titrations:

Date: (b)(4) Route: Intracranial

Number of Mice Alive

Day Post Challenge

Dilution 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 P

(b)(4)

2.15

Median mouse  
lethal dose =

(b)(4)

(b)(4)

Route: Intramuscular

Day Post Challenge

Dilution 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 P

(b)(4)

3.4

Median mouse  
lethal dose =

(b)(4)

(b)(4)

(Failure of the inoculum to induce 100% mortality at the lowest dilution tested makes calculation of the endpoint by the Spearman-Kärber method invalid.)

TABLE 3

(b)(4)

Feline Three-year Duration of (b)(4)  
 Serum Neutralization Antibody Titers ( $\log_{10}$ )  
 Vaccination Date 6 November 1982

		Months Post Vaccination								
		0	1	3	6	12	18	24	30	36
1	82MC3									
2	82MF3									
3	82MF4									
4	82MU1									
5	82MU2									
6	82MU3									
7	82MV3									
8	82MW2									
9	82MX3									
10	82MY4									
11	82MZ1									
12	82MZ2									
13	82NA4									
14	82NB2									
15	82ND5									
16	82NE3									
17	82NF5									
18	82NH1									
19	82NH2									
20	82NI5									
21	82NN5									
22	82N02									
23	82NR1									
24	82NT3									

(b)(4)

Table 3 continued

		Months Post Vaccination							
		0	1	3	6	12	18	24	30
25	82NU4	(b)(4)							
26	82NY3								
27	82NY4								
28	820A2								
29	820E2								
Mean ( $\log_{10}$ )									
GMT (reciprocal)									
All controls $\log_{10}$									
GMT (reciprocal)									

\*ND=not done

October 3, 1985

(b)(6)

Merieux Laboratories, Inc.  
117 Rowe Road  
Athens, GA 30601

Dear Mr. Hildebrand:

This will acknowledge receipt of your letter of September 3, 1985, with

(b)(4)

The research report has been reviewed and filed as satisfactory. In answer to your question concerning lowering the NIH test requirements for release of this product, the release value can not be lower than the highest value used in (b)(4). Although a NIH (b)(4) study, (b)(4) in the horse trials and therefore serial release will require use of the higher RP value.

Sincerely,

G. P. Shibley

George P. Shibley, Ph.D.  
Chief Staff Microbiologist  
Veterinary Biologics Staff  
Veterinary Services

cc:

NVSL, Ames, IA (w/cy of incom)  
VBFO, VS, Ames, IA (w/cy of incom)

APHIS:VS:GPSibley:mem:436-8674:10-2-85:mem1

*dlc*



SEP 10 1985

JET  
9/26/85

**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

3 September 1985

Dr. George P. Shibley  
USDA/APHIS/VS  
Federal Building  
6505 Belcrest road - Room 829  
Hyattsville, MD 20782

Dear Dr. Shibley:

Enclosed for your review and comments/approval are the test results of our recently completed three year immunogenicity test in dogs.

These results demonstrated [redacted] (b)(4) n vaccinates receiving one dose of vaccine with an NIH [redacted] (b)(4) in controls. The vaccinates and controls were held for 37 months post vaccination, then challenged with virulent rabies virus and held for an additional [redacted] (b)(4)

Upon review of this data we desire to discuss further the NIH requirements for serial release.

Appreciating your consideration, I remain:

Sincerely:

[redacted signature block]  
(b)(6)

can not be lower than the highest value use in immunogenicity studies  
AP=5.5 in horse study

Enclosure: 3-year Canine Immunogenicity

*Spillie  
COW*

EP 10 1985



**MERIEUX LABORATORIES, INC.**  
**VETERINARY DEPARTMENT**

30 August 1985

RABIES

3-YEAR (b)(4)

IN DOGS

PROTOCOL/RESULTS

1.0 PROTOCOL

1.1 Vaccine:

Vaccine serial (b)(4) was used to immunize the dogs in this trial. Using 16-18g mice, the NIH value was found to be (b)(4) to reach a theoretical value of (b)(4) administration. Serial (b)(4) purity, safety and potency per 9 CFR 113.129.

1.2 Dogs/Vaccination:

All were received from a beagle company at (b)(4) Inc. These dogs were subcutaneously vaccinated with one dose of vaccine on (b)(4) months of age.

1.3 Size/Sex of Challenge Groups:

27 vaccinated (16 male, 11 female) and 28 unvaccinated (12 male, 16 female) controls were challenged. Approximately 50% of the control dogs were younger than the ones vaccinated because at the time of vaccination we were not aware of the California requirements for additional controls.

1.4 Challenge Virus:

The challenge virus was a canine salivary gland suspension of (b)(4). The challenge virus was (b)(4) order to inject approximately (b)(4). The challenge virus was titered in mice to (b)(4) calculate the actual (b)(4) the virus.

(b)(4)  
3-Year (b)(4) in Dogs  
Protocol/Results  
Page 2

#### 1.5 Method/Date of Challenge:

The challenge virus was given bilaterally into the temporal muscles (b)(4). A new needle was used for each injection. A separate syringe was used for each dog. All dogs were challenged on (b)(4).

#### 1.6 Serology:

Serum virus neutralization titers were conducted on serum samples of all dogs before vaccination and in all vaccinated dogs at months (b)(4) months post vaccination and in all dogs surviving challenge; i.e., 3-months post challenge.

#### 1.7 Holding Period Following Challenge:

All challenged dogs were kept until death or for (b)(4) following challenge, whichever came first. Completion of the test was (b)(4).

#### 1.8 Confirmatory Procedures:

Brain tissues were collected from all dogs and mice dying following challenge. Impression smears of brain tissue were observed by IFA technique to confirm rabies.

#### 1.9 Incidental Deaths:

No incidental deaths occurred during the post-challenge period.

#### 1.10 General:

The vaccinates as well as controls were each housed in separate enclosures during the post challenge period so as not to distort challenge results in the control dogs.

### 2.0 RESULTS

#### 2.1 Challenge Virus Titer:

The median mouse lethal dose per (b)(4) when titered by the intracranial route (b)(4) when titered by the intra muscular route (See table 1).

(b)(4)

3-Year (b)(4) in Dogs  
Protocol/Results  
Page 3

## 2.2 Vaccination/Challenge/Rabies Confirmation:

Twenty-eight of twenty-eight (28/28) rabies susceptible controls died between (b)(4) post challenge (See table 2). Acetone-fixed impression smears from all 28 dogs were stained with a (b)(4) commercial (b)(4)

The specificity of the conjugate was confirmed by reacting it with the following control materials: impression smears prepared from normal dog brain (b)(4); brain impression smears from mice intracranially inoculated with challenge virus (b)(4); and brain impression smears from one of the susceptible dogs that died of rabies in the preliminary challenge evaluation (b)(4). Specific fluorescence for rabies viral antigen was present in impression smears prepared from the brain of each of the dead 28 control dogs.

One of twenty-seven vaccinates (1/27) (b)(4) died on day 17 post challenge. Rabies infection of the brain was confirmed by FA testing.

## 2.3 Serology:

Serological data for each dog is reported for months (b)(4) (b)(4) months post challenge (See table 3). The mean titer of the protected vaccinates was (b)(4) a range of (b)(4). The one vaccinate that died (b)(4) had the low titer of (b)(4) of (b)(4) this dog were low throughout the test for some unknown reason.

## 3.0 SUMMARY

The three year immunogenicity test in dogs was satisfactorily concluded with (b)(4) in dogs receiving (b)(4) of Merieux Rabies vaccine, killed virus with an NIH value of (b)(4). It should be noted that this vaccine antigen was (b)(4) approximately 1 year old when administered to dogs in this test.) All 28/28 unvaccinated controls died by day 17 post challenge. Rabies virus presence was confirmed by FA in the brain in all dogs that died in the test.

(b)(4)

3-Year (b)(4) in Dogs  
Protocol/Results  
Page 4

Serological antibody titers were consistently high in all vaccinates throughout the 3 years post vaccination except for dog # TBI-24-2 which consistently declined over the 37 month period to  $\leq 0.3$ . A majority of the vaccinates (17/26) responded to challenge with an increase in serum neutralizing antibody.

The study demonstrated persistent protective rabies immunity in dogs for three years post vaccination.

(b)(6)

Enclosures:

- Table 1 - (b)(4) of Challenge Inoculum in Mice  
Table 2 - Response of Dogs (b)(4) with Virulent Rabies Virus  
Table 3 - Serological Data

Table 1. (b)(4) of Rabies Virus Challenge Inoculum

Rabies (b)(4)  
 Date: (b)(4) Route: I.C.

Number of Mice Alive

Dilution 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 P

(b)(4)

Median mouse lethal dose = (b)(4) 2.0

(b)(4)

Rabies (b)(4)  
 Date: (b)(4) Route: I.M.

Number of Mice Alive

Dilution 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 P

(b)(4)

Median mouse lethal dose = (b)(4)

(b)(4)

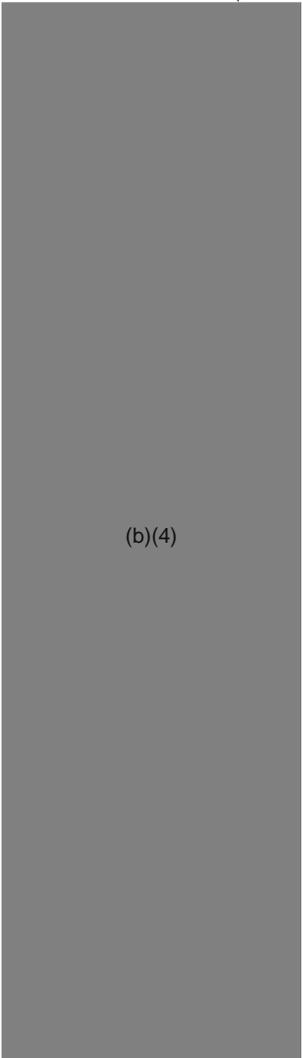
Table 2. (b)(4) Canine Three Year Duration of (b)(4) Responses of Dogs Intramuscularly Challenged 5/24/85 With Virulent Rabies Virus Inoculum.

Dog Number	Vaccination Status	Day Post Chal.		F. A. Test
		Onset of Illness	Death	
AAVCZ 634	Control			Pos.
CAHFH 3	Vaccinate			
HBVDI 4	Vaccinate			
HBXDG 1	Control			Pos.
HDHCX 314	Control			Pos.
HEKDA 4	Control			Pos.
HEL 13 1	Vaccinate			
HFZDH 4	Vaccinate			
HJMEL 1	Control			Pos.
HJP 3 2	Vaccinate			
HJPES 3	Vaccinate			
HJPES 6	Control			Pos.
HKREN 214	Control			Pos.
HMVFJ 1	Vaccinate			
HOACZ 414	Control			Pos.
HQJFK 1	Vaccinate			
HSJEZ 1	Control			Pos.
HSJFD 614	Control			Pos.
HSLFZ 714	Control			Pos.
HSNEZ 4	Vaccinate			
HTTFT 5	Vaccinate			
HUA 6 2	Control			Pos.
HUA 6 5	Vaccinate			
HUA 6 7	Vaccinate			
HUMEC 2	Vaccinate			
HUMEC 3	Vaccinate			
HUQEK 3	Vaccinate			
HUS 7 3	Control			Pos.
HVIEI 3	Vaccinate			
HVJFE 4	Control			Pos.
HWBPH 1	Vaccinate			
HWR 5 1	Vaccinate			

(b)(4)

Table 2 (Continued):

Dog Number	Vaccination Status	Day Post Chal.		F. A. Test
		Onset of Illness	Death	
HWR 5 2	Control			Pos.
HWR 5 4	Vaccinate			
HWR 5 6	Vaccinate			
HYGFO 6	Control			Pos.
HZZFD 4	Control			Pos.
HZZFD 8	Control			Pos.
RCB 5 5	Vaccinate			
RJDCH 634	Control			Pos.
RJYBX 214	Control			Pos.
RKWDG 314	Control			Pos.
RPNEF 514	Control			Pos.
RPNEF 614	Control			Pos.
RQICE 114	Control			Pos.
RRQCI 314	Control			Pos.
RSHCK 114	Control			Pos.
RWJES 314	Control			Pos.
TADFX 3	Vaccinate			
TBDEA 1	Control			Pos.
TBI 24 2	Vaccinate			Pos.
TBREN 2	Vaccinate			
TCXFW 3	Vaccinate			
TCXFW 6	Vaccinate			
TDJEA 2	Vaccinate			



(b)(4)

Pos.

TABLE 3

(b)(4)

Canine three-year duration of  
Antibody Titers ( $10g_{10}$ )

(b)(4)

serum neutralization

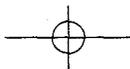
Dog Number	Months Post Vaccination									Months Post Challenge
	0	1	3	6	9	12	17	24	37	3
1 TDJEA #2										
2 HEL13 #1										
3 HVIEI #3										
8 HWR-5 #6										
10 CAHFH #3										
12 HFZDH #4										
14 TBI24 #2										
26 HJP 3 #2										
27 TBREN #2										
28 HWR 5 #4										
41 HBVDI #4										
42 HUQEK #3										
43 HMOVJF #1										
44 HQJFK #1										
45 TCXFW #3										
46 HUA 6 #7										
47 HTTFT #5										
48 HSNEZ #4										
49 HUA 6 #5										
50 HJPES #3										
51 TADFX #3										
52 HWR 5 #1										
53 HUMEC #2										
54 HWBFH #1										
55 HUMEC #3										
57 TCXFW #6										
17 RCB 5 #5										
ALL CONTROLS										

(b)(4)

FINAL CARTON LABEL  
RABIES VACCINE, KILLED VIRUS

(b)(4)

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**INDICATIONS:** Recommandé pour la vaccination des chiens, des chats et des furets en santé âgés d'au moins 12 semaines contre la maladie attribuable au virus de la rage.  
**POSOLOGIE:** Par technique aseptique, administrer 1 mL (1 dose) en injection sous-cutanée ou intramusculaire aux chiens et aux chats en santé. Revacciner un an plus tard, puis tous les trois ans. Administrer 1 mL (1 dose) en injection sous-cutanée aux furets. Revacciner annuellement les furets.  
VOIR LE MODE D'EMPLOI COMPLET AU VERSO.

MERIAL  
(Rabies 3 Year TF / Rage 3 ans TF)

Contains 50 Doses  
50 x 1 Dose (1 mL)

Contient 50 doses  
50 x 1 dose (1 mL)

Rabies Vaccine  
Killed Virus

Vaccin antirabique  
Virus tué

Rabies 3 Year TF / Rage 3 ans TF



Imrab® 3 TF

Store at 2-7°C (35-45°F).  
Do not freeze. For Veterinary Use Only.  
IMRAB is a registered trademark of MERIAL.

Entreposer entre 2 et 7 °C (35 et 45 °F).  
Ne pas congeler. Pour usage vétérinaire seulement.  
IMRAB est une marque déposée de MERIAL.

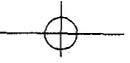
50 doses

Manufactured By / Fabriqué par  
MERIAL, INC.  
Athens, GA 30601 USA / É.-U.  
U.S. Vet. Lic. No. 298 /  
Perm. vét. des É.-U. n° 298  
1-888-Merial-1 (1-888-637-4251)

Distributed In Canada By /  
Distribué au Canada par  
MERIAL CANADA, INC.  
Baie d'Urfé, Qc. H9X 3V1  
Prod. No. / N° prod. 2311-50 -chg.



**INDICATIONS:** Recommended for the vaccination of healthy cats, dogs, and ferrets 12 weeks of age and older for prevention of disease due to rabies virus.  
**DOSAGE:** Aseptically inject 1 mL (1 dose) subcutaneously or intramuscularly into healthy cats or dogs. Revaccinate 1 year later, then every 3 years. Inject 1 mL (1 dose) subcutaneously in healthy ferrets. Revaccinate ferrets annually.  
SEE REVERSE SIDE FOR COMPLETE DIRECTIONS.



Replaces Label N

(b)(4)

see sketch 1610, 10/22/02

Bilingual English/French.

The French is a direct translation  
of the English text.

(b)(4)

- compared to sketch no. 1610

AD 01-10-02

#2 *with*

LABEL NO. (b)(4)  
USDA  
CENTER FOR VETERINARY BIOLOGICS

JAN 13 2003

LICENSING & POLICY DEVELOPMENT  
USE PERMITTED UNTIL FURTHER  
NOTICE

AL 2694 TN

FINAL CARTON LABEL

RABIES VACCINE, KILLED VIRUS

(b)(4)

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**DESCRIPTION:** IMRAB\* 3 TF contains the same virus strain that is used in the Pasteur Merieux Connaught human vaccine. The virus is grown in a stable cell line, inactivated, and mixed with a safe and potent adjuvant. Safety and immunogenicity of this product have been demonstrated by vaccination and challenge tests in susceptible animals.

**INDICATIONS:** IMRAB\* 3 TF is recommended for the vaccination of healthy cats, dogs, and ferrets 12 weeks of age and older for prevention of disease due to rabies virus.

**DOSAGE:** Aseptically inject 1 mL (1 dose) subcutaneously or intramuscularly into healthy cats or dogs. Revaccinate 1 year later, then every 3 years. Inject 1 mL (1 dose) subcutaneously in healthy ferrets. Revaccinate ferrets annually.

**PRECAUTIONS:** Store at 2-7°C (35-45°F). Do not freeze. Shake well before using. Do not use chemicals to sterilize syringes and needles. Contains gentamicin as a preservative. This product does not contain thimerosal. A transient local reaction may occur at the injection site following subcutaneous administration. Some reports suggest that in cats, the administration of certain veterinary biologicals may induce the development of injection site fibrosarcomas. In rare instances, administration of vaccines may cause lethargy, fever, and inflammatory or hypersensitivity types of reactions. Treatment may include antihistamines, anti-inflammatories, and/or epinephrine.

SOLD TO VETERINARIANS ONLY.

RM960R1

**DESCRIPTION :** IMRAB\* 3 TF renferme la même souche virale que celle utilisée dans le vaccin pour les humains de Pasteur Merieux Connaught. Le virus est propagé dans une lignée cellulaire stable, inactivé et mélangé avec un adjuvant sûr et efficace. L'innocuité et l'immunogénicité de ce vaccin ont été démontrées par la vaccination d'animaux susceptibles et par leur exposition au virus lors d'épreuves de provocation.

**INDICATIONS :** IMRAB\* 3 TF est recommandé pour la vaccination des chats, des chiens et des furets en santé âgés d'au moins 12 semaines contre la maladie attribuable au virus de la rage.

**POSOLOGIE :** Par technique aseptique, administrer 1 mL (1 dose) en injection sous-cutanée ou intramusculaire aux chiens et aux chats en santé. Revacciner un an plus tard, puis tous les trois ans. Administrer 1 mL (1 dose) en injection sous-cutanée aux furets en santé. Revacciner annuellement les furets.

**MISES EN GARDE :** Entreposer entre 2 et 7 °C (35 et 45 °F). Préserver du gel. Bien mélanger avant l'utilisation. N'utiliser aucun produit chimique pour stériliser les seringues et les aiguilles. Renferme de la gentamycine comme agent de conservation. Ce produit ne renferme pas de thimérosal. Une réaction locale transitoire peut être observée au point d'injection, à la suite d'une administration sous-cutanée. Quelques rapports indiquent que chez le chat, l'administration de certains vaccins peut provoquer la formation de fibrosarcomes au point d'injection. Dans de rares cas, la vaccination peut causer de la léthargie, de la fièvre et des réactions inflammatoires ou d'hypersensibilité. Traiter avec des antihistaminiques, des anti-inflammatoires ou de l'épinéphrine.

LA VENTE DE CE PRODUIT EST RESTREINTE AUX VÉTÉRINAIRES.

Replaces Label N

(b)(4)

see sketch 1610, 10/22/02

Bilingual English/French.

The French is a direct translation of the English text.

- compared to sketch no. 1610 (date stamped

(b)(4)

LABEL N (b)(4)  
CENTER FOR VETERINARY LOGICS

JAN 13 2003

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USE PERMITTED UNTIL FURTHER  
NOTICE

AL 2694

TN