AD-A163 084 EVALUATION OF A NEW RIFT VALLEY FEVER VACCINE: SAFETY AND IMMUNOGENICITY TRIALS(U) ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FORT DETRICK MD 1/1

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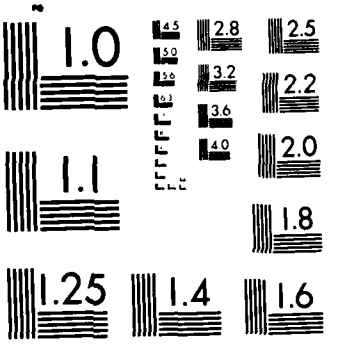

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virulence had not been preserved by conventional post-filtration or pre-inactivation virus infectivity assays, or by results of animal potency tests. These findings emphasize the need for statistically valid in vitro potency testing and the development of accurate predictive preclinical measurements for this and other vaccines.

SGRD-RMS (SGRD-UIZ-D/13 Nov 85) 1st End Mrs. Madigan/fm/AUTOVON 343-7325
SUBJECT: Clearance of Technical Manuscript

Headquarters, U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD 21701-5012

18 DEC 1985

TO: Commander, U.S. Army Medical Research Institute of Infectious Diseases, ATTN: SGRD-UIZ-D, Fort Detrick, Frederick, MD 21701-5011

The manuscript entitled "Evaluation of a New Rift Valley Fever Vaccine: Safety and Immunogenicity Trials," by Gilcin F. Meadors III, et al (encl 1) has been reviewed and is approved for publication.

FOR THE COMMANDER:

Encl
nc

LAWRENCE L. WARE, JR.
Chief
Scientific and Technical
Information Division

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FORT DETRICK, FREDERICK, MARYLAND 21701-5011

IN REPLY REFER TO

SGRD-UIZ-D

13 November 1985

SUBJECT: Clearance of Technical Manuscript

Commander
U.S. Army Medical Research and Development Command
ATTN: SGRD-RMS
Fort Detrick
Frederick, MD 21701-5012

1. The enclosed manuscript entitled "Evaluation of a new Rift Valley fever vaccine: Safety and immunogenicity trials," by Gilcin F. Meadors III, Paul H. Gibbs, and C. J. Peters, is submitted for clearance.

2. This manuscript will be submitted for publication in the journal, Vaccine.

3. This manuscript has been reviewed by members of the staff of this Institute and clearance is recommended.

Encl

Reviewed: [illegible]

/s/
DAVID L. HUXSOLL
Colonel, MC
Commanding

EVALUATION OF A NEW RIFT VALLEY FEVER VACCINE: SAFETY AND IMMUNOGENICITY TRIALS[1,2]

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FOOTNOTES

[1]The subjects in these studies were United States Army personnel at the United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD. The studies were governed by the principles, policies, and rules for medical volunteers as established by Army Regulation 70-25 and the Declaration of Helsinki.

[2]The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Key words: Rift Valley fever, vaccine, safety, immunogenicity, inter-lot variation

Abstract

A formalin-inactivated Rift Valley fever vaccine prepared in primary monkey kidney cells has been used to protect laboratory workers from disease since 1967. A similar but improved vaccine was prepared in 1978-1979 using well-characterized diploid fetal rhesus lung cells. In initial clinical trials reported here, the new vaccine elicited high levels of plaque neutralizing antibodies and caused only minimal local reactions at the injection site. Significant variability was observed in the geometric mean titer evoked by various vaccine lots. This variability had not been predicted by conventional pre-filtration or pre-inactivation virus infectivity assays, or the results of animal potency tests. These findings emphasize the need for statistically valid human potency testing and the development of accurate predictive preclinical measurements for this and other vaccines.

Introduction

Rift Valley fever (RVF), a virus disease of man and domestic animals, has been known in sub-Saharan Africa since 1931 (1). The virus causes a serious epizootic disease of sheep and cattle, although in humans it typically results in a temporarily incapacitating but self-limited febrile illness (2). RVF virus (RVFV) has been responsible for frequent laboratory infections (3,4) and has also been associated with ocular disease, encephalitis, and hemorrhagic fever (2). The major mode of transmission, and indeed, the reservoir are thought to be the mosquito (5). However, the virus is also infectious by aerosol, a suspected route of infection in both laboratory and epidemic settings (6,7,8). In 1977 RVF was diagnosed in Egypt for the first time. The subsequent epidemic had a high attack rate in the unprotected and previously unexposed population of the Nile Valley, and unprecedented numbers of people developed the serious complications that had previously been reported on a smaller scale (9). Thus, the virus not only causes lethal human disease, but also can spread to receptive areas outside its established geographical range (10).

Since 1967, a formalin-inactivated vaccine produced from RVF virus propogated in infected primary monkey kidney cells (NDBR-103) has been used to protect laboratory workers (11,12)[1]. Over 2000 persons have received this vaccine, including over 1000 members of the Swedish peacekeeping forces stationed in the Sinai peninsula during the 1977 Egyptian epidemic (13). In over 95% of the recipients, the vaccine induced antibody titers thought to be protective (14)[2]. Laboratory workers with vaccine-induced antibodies have not

[1]Annual Report from USAMRIID to FDA on NDBR 103, 1968-1984.

[2] G. F. Meadors, unpublished observations.

developed overt illness while working with the virus; however, serological monitoring has detected asymptomatic rises in antibody titers[1].

Adverse reactions, although uncommon, include erythema and rarely induration at the site of injection (13)[2]. In the Swedish military vaccinees, a single case of Guillain-Barre syndrome occurred (13). Given the numbers of recipients needed to establish a correlation between vaccination and the Guillain-Barre syndrome (15,16), this case cannot be conclusively linked to the vaccination.

In spite of the safety and success of the old vaccine, it was prepared with methods that are not considered optimal by today's standards. The vaccine seed virus inoculum was composed of infectious mouse serum and primary monkey kidney cells were the substrate. Therefore, the original seed virus was cloned and propagated in cell culture, and a well-characterized, diploid rhesus monkey cell substrate[3] was utilized to prepare the new vaccine. Twenty lots of new vaccine were manufactured in 1978 and 1979. This paper reports results of the initial clinical trials of this vaccine.

Materials and methods

Vaccine

Rift Valley Fever Vaccine, Inactivated, Dried, TSI-GSD-200 (lots 1 through 8), was obtained from the manufacturer, The Salk Institute, Government Services Division, Swiftwater, PA. It was stored at -20 °C, reconstituted with 5.5 ml sterile water for injection and used within two hours of reconstitution. Excess reconstituted vaccine was destroyed by autoclaving.

[1]G. F. Meadors, unpublished observations.

[2]Annual Report from USAMRIID to FDA on NDBR 103, 1968-1984.

[3]IND submission, TSI-GSD-200.

Volunteers

A total of 52 volunteers, males and females, ages 18-65, were recruited for these trials. Informed consent was obtained from all volunteers. The studies were conducted under an investigational new drug application granted by the Food and Drug Administration.

Prospective volunteers underwent a complete history, physical examination, and laboratory testing, including urinalysis, complete blood count (CBC), serum chemistry panel, chest roentgenography, and electrocardiography. Volunteers were accepted for study only if no significant abnormalities were found by these screening procedures.

Experimental design

Volunteers were assigned to experimental groups sequentially in the order in which they volunteered. The clinical trials involved four separate protocols.

In the first protocol, six volunteers who had previously received the old vaccine were injected with either 0.1 ml intradermally or 1.0 ml subcutaneously (s.c.) of vaccine from lot 1, run 1. Two of these subjects (two and six) worked daily with the virus. Serological response was measured on days 0, 7, and 14 post-vaccination.

In the second protocol, five volunteers who would not be exposed to the virus, but who had participated in studies of the old vaccine, were injected s.c. with 1.0 ml of the new vaccine from lot 1, run 1, and were assayed serologically on days 0, 7, 14, 21, 28, 35, 42, 91, and 182.

In the third protocol, 13 volunteers who had not previously received the old vaccine and would not be exposed to the virus were injected s.c. with 0.1,

0.3, or 1.0 ml of the vaccine from lot 1, run 1, on days 0, 10, and 28 and were assayed for serological response on days 0, 7, 14, 21, 28, 35, 42, 91, 182, 273, and 364. In some cases, serum from day 4 was also assayed for early appearance of antibodies.

In the fourth protocol, 28 volunteers were injected s.c. on days 0, 10, and 28 with 0.3 ml of vaccine from one run of each of lots 2 through 8 of the vaccine. The volunteers were recruited in separate groups for each lot and vaccinated as the lots became available for human use.

Clinical assessment

Volunteers were observed for 30 min following vaccination for any immediate reaction. They were instructed to report to the Clinical Research Ward at any time for documentation and treatment of any local or systemic reaction, including fever. Urinalysis, CBC and serum chemistries were obtained on days 7, 14, and 35.

Serologic response

The immunologic response of the volunteers was assessed by means of an 80% plaque reduction neutralization (PRNT80) assay resembling that used by Kark, Aynor, and Peters (17). Briefly, sera in two-fold dilutions were mixed with approximately 80 to 120 plaque forming units (PFU) virus to yield final dilutions ranging from 1:5 to 1:1280. After incubation at 37°C for one hour, residual virus was assayed in duplicate VERO cell monolayers in 60 mm wells. The highest dilution of serum reducing the virus titer by 80% or more was deemed to be the PRNT80. Appropriate controls were included in each test, and after the completion of protocols three and four, all sera from each post-vaccination day from 7 to 42 were retested in a single assay.

Tests for viremia

In protocols three and four, sera for virus assay were obtained on days two, three, and four post-vaccination and stored at -70°C until assayed. In protocol three, 80 to 100 g male Syrian hamsters (Mesocricetus aureatus) (Charles River Laboratories, Cambridge, MA) were given intraperitoneal injections of 1.0 ml of serum. The hamsters were then observed for seven days for mortality. In protocol four, 1.0 ml aliquots of sera were assayed for PFU in VERO cell monolayers.

Preclinical assay

The pre-filtration virus titer was measured as PFU in VERO cell monolayers. The pre-inactivation titer was determined as 50% lethal doses (LD_{50}) after intraperitoneal inoculation of adult mice ($MIPLD_{50}$). Post-inactivation potency was expressed in terms of the effective vaccine dose needed to protect 50% of mice against challenge with virulent virus (ED_{50}). Both the LD_{50} and ED_{50} were estimated by the Reed-Muench method[1]. These test were performed by the Salk Institute, Government Services Division, Swiftwater, Pa.

Statistical methods

For purposes of analysis, the PRNT80's and vaccine doses were transformed to base 10 logarithms. The inter-lot and intra-lot variabilities in PRNT80's were quantified by analysis of variance techniques followed by Tukey-Kramer multiple comparisons to partition the lots into groups (18). The Tukey-Kramer method has been shown to have superior power characteristics compared to other procedures (19). The relative responses to the various lots were estimated by

[1]IND Submission for NDBR 103.

the ratio of the geometric mean titers, and 95% confidence intervals were estimated for the ratios. Lots which were statistically indistinguishable exhibited 95% confidence intervals which included unity.

Regression techniques (18) were used to estimate dose response curves. Calculations were performed to assess the number of volunteers required to detect a twofold difference in inter-lot relative response with 85% power, and to construct a dose-response curve with 95% significance.

Results

Reactions to vaccination were mild and consisted of local reactions limited to areas of erythema less than 2 cm in diameter. No fever or other systemic reaction occurred, and no significant clinical laboratory abnormalities were observed. No viremia was detected at any time.

Serological responses to vaccination as measured by the PRNT80 are tabulated in Tables 1-3. Three of the 45 previously unimmunized volunteers had pre-immunization PRNT80's of 1:5. None had a history that suggested previous exposure to a Phlebovirus. Their responses were indistinguishable from those of the other 42 previously volunteers whose PRNT80 was $< 1:5$ and presumably reflect a non-specific suppression of viral infectivity.

Previously vaccinated volunteers who received 0.1 ml of vaccine intradermally developed no booster response. Substantial increases in titer were observed in those receiving 1.0 ml subcutaneously (Table 1).

Of the volunteers in protocols 3 and 4 (Tables 2 and 3) (all of whom were non-immune) only 1/41 developed a titer $\geq$ 1:40 on days 4 or 7. Three of four recipients of 1.0 ml doses of vaccine (Table 2), 7/19 recipients of 0.3 ml doses (Table 3), and 1/3 recipents of 0.1 ml doses (Table 2) developed titers $\geq$ 1:40 as early as day 14. For all doses of vaccine, maximum titers were

observed on days 35 and 42. In volunteers who received 0.3 ml doses, 29/31 tested on day 35 and 23/28 tested on day 42 developed titers $\geq$ 1:40. Six of 16 tested on day 182 and 6/19 tested on day 364 still had titers $\geq$ 1:40.

Analysis of the data for days 35 and 42 for lot 1 (Table 2) suggested a linear relation between log dose and log mean titer. The slopes of the regression lines were .80 ± .46 for day 35 and .90 ± .48 for day 42. About 26% of the variation is accounted for by the linear trend, which, however, is not statistically significant at the 95% level.

We found significant inter-lot variability in the serological responses to 0.3 ml doses of lots 1-8 on days 35 and 42 (analysis of variance $p <$ .004). On both days, the ranking of lots was identical except for lot 7 and about 60% of the variance was due to inter-lot differences. Further analysis by the Tukey-Kramer method separated the responses to lot 4 from responses to lots 1, 2, 3, and 7 for day 35, and the responses to lots 4 and 5 from those of lots 1 and 3 on day 42 (Table 4).

For day 35, there was a 19-fold difference (1.28 logs) in the observed geometric mean titers in response to vaccination with the polar lots, lot 4 and lot 7. The 95% confidence interval for the difference in responses to lot 4 and lot 7 was 4.8 to 77-fold. The smallest observed difference which would be significant at the 95% confidence level was 0.93 logs, or an 8.4-fold difference in the geometric mean titer. For day 42, the relative responses to the extreme lots, lot 4 and lot 3, was 21-fold (1.33 logs). The 95% confidence interval for the difference in responses to lot 4 and lot 3 was 3.7 to 125-fold. The smallest observed difference which would be significant at the 95% confidence level was 1.25 logs, or an approximately 17.8-fold difference in the geometric mean titer. The intra-lot variability, estimated for use subsequent in power calculations, was 0.38 logs for day 35 and 0.48 logs for day 42.

No correlation was found between any of the data supplied by the manufacturer and the measured human response (Table 5). Indeed, there was little correlation (r=.19) between the pre-filtration PFU titer and pre-inactivation mouse LD_{50}. The correlation between the mouse ED_{50} and the human log mean titer for each lot was estimated to be -.06 for day 35 and -.01 for day 42.

Discussion

RVF is a significant human and domestic livestock disease of sub-Saharan Africa and has demonstrated its potential for distant spread (10). Since control of zoonotic amplification and secondary spread to man will prove to be difficult if RVFV is introduced into a receptive area (20), development of a successful human immunogen was particulary important (11). The original formalin-inactivated cell culture derived vaccine has now been superceded by a similar product that uses a more acceptable seed virus and a well-characterized diploid cell substrate.

Our initial clinical trials of this new vaccine in man were promising. In 52 volunteers the only adverse effects observed were local erythema at the site of injection. Systemic reactions or laboratory abnormalities did not occur. No viremia was detected in volunteers, confirming the negative pre-clinical tests for residual live virus in the vaccine.

Furthermore, the vaccine successfully induced virus-neutralizing antibodies. Peak PRNT80 titers often exceeded 1:1280 and antibody persisted in most subjects available for 9 to 12 month follow-up. Titers of 1:40 or greater were measured at least once in all recipients of 1.0 ml doses and in 30/31 of the recipients of 0.3 ml doses. This response rate appears similar to that of the old vaccine. The PRNT80 titers of 1:5 found in 6.6% of

previously unimmunized volunteers probably represent non-specific plaque suppression, a phenomenon often seen when low dilutions of serum are used in such tests. We included measurements at the 1:5 level to allow detection of early signs of the immune response in this study, but would use 1:10 as an initial dilution in most situations.

The significance of these neutralizing antibody titers in protecting man is not known. There is considerable evidence in experimental infections of rodents and primates (21)[1] that antibody is the major immune effector mechanism responsible for recovery from RVF and can protect against disease even in very low titers. Induction of antibodies by vaccination of rodents has correlated with survival from virus challenge (11) and, indeed, passively transferred neutralizing antibody titers afford roughly the same protection as those acquired by vaccination (14). At USAMRIID, a PRNT80 of 1:40 or greater is required before entry into the P-3 containment laboratories where the virus is under study. Using this standard no laboratory acquired RVF has been observed at USAMRIID, although numerous laboratory infections have been reported in unimmunized laboratory workers elsewhere. Thus, use of serum neutralizing titers to monitor human protection is conceptually reasonable and has proven effective. The 1:40 level chosen is safe, although probably conservative.

In light of the vigorous immune response to this vaccine, the dose recommended for its predecessor (1.0 ml on days 0, 7, and 28) may be too high for at least some of the lots of the new vaccine. To assess the possibility that a smaller dose might suffice we need a more precise estimate of the function describing the response to graded doses of vaccine, the mean response

[1] C.J. Peters, unpublished observations.

for a given dose of each of the lots, and the variance of the response. Analysis of the data from this trial and that presented by Kark, Aynor, and Peters (17) suggests that the expected log dose-log response function will apply (calculations not shown), although the small sample sizes used and the large variance of the human immune response limit the strength of this conclusion. Interestingly, and perhaps fortuitously, the day 42 figures from both human studies, as well as the hamster data (14), suggest a linear relation between log-dose and log-PRNT80 with slope 1 and intercept at the origin. If we assume the linear model and the 0.48 intralot variance observed in this study, 37 subjects per vaccine dose would be required to detect a two-fold difference in potency with 80% power and 95% significance to halve vaccine doses.

Even with the small number of subjects used in this study, we detected significant inter-lot variations in potency. Differences of this magnitude are both statistically and clinically significant. They have impact upon determining the minimum effective dose of the vaccine. Furthermore, they are of significance to control of the manufacturing process. With present methodology, neither virus infectivity determined before and after filtration nor final product antigenicity assayed in mice predicted the inter-lot variability of the human response. Ongoing studies suggest that this failure may be the result of the characteristics of the Reed-Muench statistic for estimating the ED_{50} and LD_{50}, the numbers of test animals or cell culture replicates employed in the assays, and the dilution series chosen for testing (Meadors, unpublished data).

The variations in potency of the RVF vaccine are not an isolated phenomenon in vaccine manufacture. For example, a vaccine for a totally

unrelated virus was recently withdrawn from the market (22)[1] because of failure of similar pre-clinical trials to predict human response.

In summary, the new RVF vaccine produced no serious adverse effects, and evoked acceptable antibody responses. The significant inter-lot variation demonstrated in the present study identified future research needs to standardize production and ascertain optimal preclinical tests to predict the human immune response to this vaccine.

[1]Letter to practitioners from Wyeth Laboratories, dated 16 Feb 1985, recalling Wyvac® Rabies Vaccine.

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Table 1 PRNT80 titers in volunteers previously immunized with RVF vaccine (NDBR 103) and subsequently boastered with single doses of TSI-GSD-200, lot 1, run 1 (protocols 1 and 2)

	DAY					
SUBJECT	0	7	14	21	28	182
	PRNT80 TITERS FOLLOWING A 0.1 ML INTRADERMAL DOSE:					
1	80	40	nd[a]			
2	2560	2560	2560			
	PRNT80 TITERS FOLLOWING A 1.0 ML SUBCUTANEOUS DOSE:					
3	10	80	640			
4	20	1280	1280			
5	80	80	640			
6	160	160	320			
48	40	nd	>1280	>1280	nd	320
49	80	nd	>1280	nd	1280	320
50	320	1280	>1280	>1280	640	nd
51	20	1280	nd	>1280	640	160
52	80	640	>1280	>1280	1280	320

[a]nd=Not done; volunteer absent from USAMRIID.

Table 2 PRNT80 titers in previously unimmunized volunteers vaccinated with various doses of TSI-GSD-200, lot 1, run 1, on days 0, 10, and 28 (protocol 3)

	DAY										
SUBJECT:	0	4	7	14	21	28	35	42	91	182	272
	PRNT80 TITERS FOLLOWING 1.0 ML S.C. DOSES:										
7	5	5	20	160	80	320	320	160	80	20	nd
8	5	10	10	20	40	80	40	80	80	40	nd
9	<5	<5	20	40	640	320	1280	1280	160	80	40
10	<5	<5	5	80	320	nd	nd	nd	na[a]	na	na
	PRNT80 TITERS FOLLOWING 0.3 ML S.C. DOSES[b]:										
11	<5	80	>20	80	160	320	320	160	80	40	nd
12	<5	<5	5	20	40	20	80	160	40	40	40
13	<5	5	<5	<5	5	5	40	20	5	<5	nd
14	<5	<5	<5	5	80	20	80	20	20	5	nd
	PRNT80 TITERS FOLLOWING 0.1 ML S.C. DOSES:										
15	<5	<5	20	80	80	160	160	160	40	40	nd
16	5	<5	5	5	5	10	5	10	5	<5	<5
17	<5	<5	20	20	160	320	160	320	80	80	nd
18	<5	<5	5	nd	10	10	20	10	20	20	nd
19	<5	<5	<5	nd	10	10	nd	5	5	nd	nd

[a]na= Not analysed, subject received third injection on day 56.

[b]The data for lot 1 run 1, 0.3 ml doses, subjects 11-14 are also included in table 3.

Table 3 PRNT80 titers of volunteers vaccinated with 0.3 ml of lots 1-8 of TSI-GSD-200 on days 0, 10, and 28 (protocol 4)

SUBJECT	DAY 0	4	7	14	21	28	35	42	91	182	273	364
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 1 RUN 1:											
11	<5	80	>20	80	160	320	320	160	80	40	nd	nd
12	<5	<5	5	20	40	20	80	160	40	40	40	nd
13	<5	5	<5	<5	5	5	40	20	5	<5	nd	nd
14	<5	<5	<5	5	80	20	80	20	20	5	nd	nd
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 2 RUN 2:											
20	<5	<5	5	10	40	40	160	320	20	nd	nd	40
21	<5	<5	5	nd	80	160	320	320	40	nd	nd	5
22	<5	<5	<5	20	40	40	160	320	80	nd	nd	20
23	<5	5	<5	160	160	80	160	160	40	nd	nd	20
24	<5	<5	<5	5	nd	nd	<20	20	10	nd	nd	5
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 3 RUN 1:											
25	<5	<5	5	5	20	40	80	80	20	nd	nd	20
26	<5	5	5	5	20	nd	80	40	20	nd	nd	5
27	<5	5	5	20	40	80	40	80	20	nd	nd	5
28	<5	5	10	10	40	40	160	20	<20	nd	nd	5
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 4 RUN 2:											
29	<5	nd	5	320	nd	640	1280	1280	nd	nd	80	nd
30	<5	<5	20	40	640	320	640	320	160	80	40	nd
31	<5	nd	5	80	nd	320	>1280	>1280	nd	nd	20	nd
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 5 RUN 1:											
32	<5	<5	10	40	640	nd	160	1280	160	nd	nd	40
33	<5	<5	5	10	640	320	640	640	40	nd	nd	nd
34	<5	5	10	20	320	640	1280	1280	nd	nd	nd	nd
35	<5	nd	20	80	320	320	320	na[a]	na	na	na	na
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 6 RUN 2:											
36	<5	<5	<5	nd	nd	nd	320	nd	nd	40	nd	40
37	<5	<5	10	nd	nd	nd	640	160	nd	20	nd	40
38	<5	<5	10	nd	nd	nd	640	640	nd	160	nd	80
39	<5	<5	<5	nd	nd	nd	320	640	nd	40	nd	20
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 7 RUN 1:											
40	<5	<5	5	nd	nd	nd	20	40	nd	10	nd	10
41	<5	nd	<5	nd	nd	nd	80	10	5	5	nd	nd
42	<5	nd	10	nd	nd	nd	80	640	nd	20	nd	10
43	<5	<5	nd	nd	nd	nd	160	nd	nd	nd	nd	160
	PRNT80 TITERS FOLLOWING VACCINATION WITH LOT 8 RUN 2:											
44	<5	nd	nd	nd	nd	nd	320	640	160	20	nd	nd
45	<5	nd	nd	nd	nd	nd	160	320	nd	40	nd	nd
46	<5	<5	5	nd	nd	nd	320	320	nd	20	nd	20
47	<5	<5	5	nd	nd	nd	80	nd	nd	10	nd	5

a na=Not analysed: subject received only two injections.

Table 4 Tukey-Kramer multiple comparison tests on log mean titers at the 95% confidence level

DAY 35			DAY42		
LOT	LOG MEAN TITER	GROUP*	LOT	LOG MEAN TITER	GROUP*
4	3.11	A	4	3.01	A
5	2.71	A B	5	3.01	A
6	2.65	A B	6	2.61	A B
8	2.28	A B	8	2.61	A B
2	2.02	B	2	2.21	A B
1	1.98	B	7	1.80	A B
3	1.90	B	1	1.75	B
7	1.83	B	3	1.68	B

* The Tukey-Kramer test, as applied to these data, assigns each lot to one or two groups, A and/or B. Lots assigned to only one group are statistically separable from lots assigned soley to the other group. Lots assigned to both groups A and B cannot be unambiguously assigned to either group A or B.

Table 5 Comparison of preclinical assays with measured human antibody response

LOT	RUN	PFU/ML[1] x10^6	MIPLD$_{50}$[2] x10^6	ED$_{50}$[3]	LOG MEAN TITER[4]	
					DAY 35	DAY 42
1	1[5]	4.0	0.19	0.005	1.98	1.75
	2			0.004	-	-
2	1	7.8	1.00	0.005	-	-
	2			0.004	2.02	2.21
3	1	10.0	4.47	0.005	1.90	1.68
	2			0.006	-	-
4	1	8.2	0.63	0.006	-	-
	2			0.008	3.11	3.01
5	1	9.4	2.00	0.008	2.71	3.01
	2			0.003	-	-
6	1	5.5	2.75	0.011	-	-
	2			0.006	2.65	2.61
7	1	8.4	1.38	0.006	1.83	1.80
	2			0.018	-	-
8	1	5.0	3.89	0.011	-	-
	2			0.005	2.28	2.61

[1]Pre-filtration titer measured in cell culture, from manufacturer.

[2]Pre-inactivation titer as measured by mouse lethality, from manufacturer

[3]Post-inactivation mouse protection titer, from manufacturer.

[4]Log mean PRNT80 titer of human volunteers receiving 0.3 ml of vaccine on days 0, 10, and 28.

[5]Each lot of vaccine after inactivation was lyophilized in two separate sub-lots, thus creating run 1 and run 2 of each lot of final product.

Running head: Rift Valley Fever Vaccine

END

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