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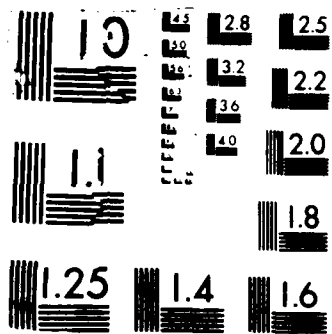
COMPARATIVE INFECTIVITY DETERMINATION OF CANDIDATE LIVE 1/1
DENGUE VIRUS VACC (U) PUERTO RICO UNIV SAN JUAN
E KRAISELBURD 27 JUL 87 DAND17-84-C-4197

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DEPARTAMENTO DE MICROBIOLOGIA

AD-A183 804

July 27, 1987

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Dear Sirs:

Enclosed please find the original and 12 copies of the Final Report of contract No. DAHD-17-84-C-4197. Please, kindly return the original to my attention.

Sincerely,

Edmundo Kraiselburd, Ph.D.
Associate Professor
Principal Investigator

sen

Enclosure

CC: Dr. José H. Saldaña, Chancellor

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Dr. Ken Eckels, Project Officer, WRAIR

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Comparative Infectivity Determination
of Candidate Live Dengue Virus Vaccine
in Monkeys, Mosquitoes and cell cultures

Annual and Final Report

Edmundo Kraiselburd, Ph.D.

May 1987

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-C-84-C-4197

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Comparative Infectivity Determination of Candidate Live Dengue Virus Vaccine in Monkeys, Mosquitoes and Cell Cultures.		5. TYPE OF REPORT & PERIOD COVERED June 1, 1984 - May 31, 1987
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Edmundo Kraiselburd, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-84-C-4197
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Puerto Rico Medical Sciences Campus San Juan, P.R. 00936		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Ms. Diana Clark US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release, distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Dengue-1 (TP79-56); Dengue 1 (45AZ-5); Dengue 3 (CH53489) and Dengue-4 (341750 Carib) vaccines. Rhesus monkeys immunity. Mosquito infectious dose 50.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The following live, attenuated dengue virus vaccines strains were tested for their respective immunogenicity in rhesus monkeys: dengue-1 (TP79-56, code TSI-GSD216 and 45AZ-5, code No. TSI-GSD214); dengue 3 (CH53489, code 24/28); and dengue-4 (341750 Carib). Clone It was found that 417 MID ₅₀ (mosquito infectious dose 50) and 116 MID ₅₀ were required for seroconversion of 50% of the animals with the 45AZ5 and the TP79-56 vaccine, respectively. Upon challenge with the homologous wild type virus,		

Accomplishments:

a) Dengue-1 vaccine studies:

A group of 32 tuberculin-negative juvenile rhesus monkeys were pretested for HI as well as for neutralizing antibodies against dengue virus serotypes I to IV. All animals were found to be free of dengue virus antibody. All monkeys were housed at the Caribbean Primate Research Center in individual steel squeeze cages in a mosquito-proof area. Fifteen days later, all monkeys were tested again (by HI tests) for the presence of dengue antibodies. Again, all animals were found to be free of antibodies against all four dengue serotypes.

On June 21, 1964 were received: four vials of the live attenuated dengue-1 vaccine strain TP79-56 (code No. TSI-GSD216) as well as of the dengue-1 vaccine strain 45AZ-5 (code No. TSI-GSD214). A bottle of vaccine diluent (FHEM with 0.25% HSA) was also received from WRAIR. Each vial of lyophilized vaccines were resuspended in 3 ml of double distilled H₂O. On vaccination day, the TP79-56 seed had a titer of 5.3×10^3 pfu/ml (9.6×10^5 mosquito infectious dose per ml, MID₅₀/ml). The 45AZ-5 vaccine had a titer of 6.3×10^3 pfu/ml (2.4×10^6 MID₅₀/ml).

Animals were divided into 2 groups of 15 monkeys each. One group received the TP79-56 vaccine and the other group was inoculated with the 45AZ-5 vaccine. On July 12, 1964 monkeys were inoculated subcutaneously with 0.5 ml of serially diluted preparations of either TP79-56 or 45AZ-5 vaccines. The highest doses used were 1,320 MID₅₀ and 322 MID₅₀ for the 45AZ-5 and the TP79-56 vaccines, respectively. Appropriate 10 fold dilutions were made in the diluent medium provided by WRAIR. Groups of 5 animals were inoculated with each vaccine dilution. Two monkeys were used as the unvaccinated controls.

Monkeys were bled on post vaccination days 30, 60, 90, 120, 150 and 180 and HI titers against dengue-1 virus were obtained following standard procedures. The virus used for HI tests was received from WRAIR on January 27, 1964 and was passed three times in Albo and 3 times in TRA 234 cells. The titer of the virus was 3.5×10^5 pfu/ml.

Results obtained are shown on tables 1 and 2. It can be seen from these tables that it required 417 MID₅₀/0.5 ml and 116 MID₅₀/0.5 ml for seroconversion of 50% of the animals with the 45AZ-5 and the TP79-56 vaccine, respectively. These doses are between 40 to 10 times higher than the dose required to infect 50% of the monkeys with either (wild type) dengue-2 or dengue-4 virus (see quarterly report 1963; Kraiselburd et al, Transactions of the Royal Society for Experimental Medicine and Hygiene, 72: 248-251, 1965). Based on this data, it would appear that the 45AZ-5 vaccine might be 3 times more attenuated than the TP79-56 vaccine.



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On day 190 post-infection, all animals were inoculated s.c. with 0.5 ml containing 2916 MID₅₀ (29.2 pfu) of DEN-1 virus (strain WRAIR - WP1974). Virus was received from WRAIR on DEC. 20, 1984 and was stored at -70°C upon arrival. For animal inoculation, virus was diluted in MEM containing 0.25% HSA.

Animals were bled on post infection days 10, 30 and 61. N tests were performed following standard procedures, using the WP1974 DEN-1 strain. Results are shown on Tables 3 and 4.

As expected, all control animals seroconverted on or before post challenge day 30 (see table IV). Dengue-1 immune animals showed a typical secondary antibody response.

b) Dengue-3 vaccine studies:

The second phase of this contract started on June 1, 1985. Its objective was to compare immunogenicity of Army's candidate Dengue-3 vaccine (CH53489) with the wild type Dengue-3 virus (strain CH53489).

On May 23, 1985 we received from WRAIR 6 vials of each, the lyophilized wild type Dengue-3 virus as well as the vaccine. Virus was labelled: CH53489, Mfg date: April 1984, lot No. 1. Vaccine was labelled: live attenuated CH53489 clone 24/28 Mfg date: Sept. 1983, lot No. 1.

On May 31, 1985 two vials of each preparation were reconstituted with 3 ml of double distilled, tissue culture-grade water. Vials were pooled and assayed for both, plaque forming units (pfu) (using the LLC-MK cell assay at 31°C) and for mosquito infectious doses (by mosquito inoculation). Titers obtained were as follows:

Vaccine:	4.85×10^2 pfu/ml	(2×10^6 MID ₅₀ /ml)
Virus :	5.25×10^2 pfu/ml	(2×10^5 MID ₅₀ /ml)

Pfu titers were read on June 6. MID₅₀ titers were reported to us by Dr. Gubler (San Juan Laboratories) on June 24.

On May 15, thirty three monkeys were housed in individual cages in a mosquito-proof area. They were bled on the same day, and their serum samples were found to be free of antibodies to the four dengue serotypes (by HI and N tests). On June 1, the animals were bled again. Serological tests (HI and N) showed no evidence of flavivirus infection. The viruses used in N tests were: D1 (WRAIR) p#3 ALBO, p#4 TRA; D2 PR459 P#2 ALBO; D3 CH53489 p#1 TRA 284, and D4 H241 (San Juan Laboratories) p#1 ALBO. The titers of the virus (as well as of the vaccine) reported above were used to calculate the dilutions required to infect groups of 5 monkeys with 500, 50 and 5 MID₅₀ of each preparation. Preparations were diluted in EMEM containing .25% HSA (sent to us by WRAIR).

On July 10, groups of 5 monkeys were either s.c. infected or vaccinated with 0.5 ml of the doses that were calculated as described above. Control monkeys received diluent only. However, vaccine as well as virus titrations performed on vaccination day, revealed a difference with the titers obtained on June 6 and 24. The new titers obtained (July 10 titers) were as follows:

Vaccine: 4.85×10^3 pfu/ml (1.6×10^7 MID₅₀/ml)

Virus : 1.05×10^3 pfu/ml (6×10^6 MID₅₀/ml)

Therefore, there was almost a 10 fold difference with the previous titers. At this time, we do not know the cause(s) of this differences (upon reconstitution both vaccine and virus vials showed always the characteristic pink color).

Based on the titers obtained with the inoculum used on vaccination day, we calculated that the monkeys were vaccinated with 4000, 400 and 40 MID₅₀, respectively. The doses of wild type virus administered to the animals were: 15,000, 1,500 and 150 MID₅₀ per 0.5 ml, respectively.

Monkeys were bled on days 30 and 60 post inoculation. Serum samples were assayed for antibodies against Dengue-3 virus (strain CH5349 p#1 TRA 284). Results obtained are shown on tables V and VI. It can be seen from these tables that both, vaccine as well as virus, were highly immunogenic. However, with the doses used, end points could not be obtained. These results were in contrast with the experimental data obtained in this laboratory with other dengue strains and vaccines, which are summarized in table VII.

Upon consultations with the project officer of this contract, Dr. Ken Eckels, it was decided to introduce 20 additional monkeys to the project and to release the animals which were infected or vaccinated with the highest doses. This modification to our original contract was decided in order to obtain end points for both, vaccine and virus. On October 28, 1985 we introduced and bled 20 additional monkeys. These animals were bled again on Nov. 12, 1985. All animals were found to be free of dengue-3 antibodies (N test performed against Dengue 3 CH53489 p#1 TRA 284).

The last two vials of vaccine and of virus were pooled separately. Appropriated dilutions were made to infect groups of 5 monkeys with 15 and 1.5 MID₅₀ of dengue-3 virus and 10 and 2.5 MID₅₀ of vaccine, respectively. The dilution factors were calculated on the basis of the titers obtained on July 10, 1985. However, the titers reported to us by Dr. Gubler varied somewhat from the July 10 titers. Titrations obtained were as follows:

Vaccine: 4.63×10^3 pfu/ml (2×10^7 MID₅₀/ml)

Virus : 8.33×10^2 pfu/ml (1.5×10^6 MID₅₀/ml)

Based on these titrations, it was calculated that the vaccinated monkeys received 12.5 and 3.125 MID₅₀, respectively. The infected monkeys received 3.75 and 0.375 MID₅₀ of dengue-3 virus, respectively. Animals were bled on post inoculation days 30 and 60. N testes were performed against Dengue-3 CH53439 p#1 TRA 284). It can be seen in tables VIII and IX that end points could not be reached with the dilution used. Thus with the lowest dose of virus and vaccine used (0.375 MID₅₀ of virus and 3.125 MID₅₀ of vaccine) 4/5 and 3/5 monkeys seroconverted, respectively.

To reach the desired end points, virus preparations were further diluted and inoculated into experimental animals (under contact extension). New monkeys were bled and their serum samples were assayed by HI and N tests (against the four dengue serotypes) for any evidence of previous dengue virus infection. All animals were found to be free of flavivirus antibodies and were brought to a mosquito-proof area.

Fifteen days later, animals were bled again and their serum samples assayed for dengue antibodies, as described above. No evidence of dengue antibodies was found. Dengue 3 vaccine (CH53489) and virus were titrated in mosquitoes and in cell culture using standard procedures. Titers were: 9.6×10^6 MID₅₀/ml and 4.54×10^3 pfu/ml (vaccine) and 1.5×10^6 MID₅₀/ml and 4×10^3 pfu/ml (virus).

Preparations were titered and diluted on vaccination day (March 13, 1986). A group of monkeys was s.c. inoculated with 0.3072 MID of vaccine. The other group of monkeys received 0.0297 MID of wild type virus. Both inocula were delivered in 0.5 ml of diluent (EMEM, 0.25% HSA) sent to us by Dr. Ken Eckels.

Animals were bled on post infection days 30 and 60 and their serum samples assayed for N antibodies against Dengue-3 (wild type) virus. Results obtained are shown in table X and XI. It can be seen from these tables that with the doses used, no seroconversions were obtained. These data allowed us to calculate the MID₅₀ required to infect rhesus monkeys. Using the method of Reed and Muench, the following MID₅₀ were obtained.

Dengue-3 vaccine = 2.93 MID₅₀

Dengue-3 virus = 0.158 MID₅₀

These results showed that the dengue-3 virus was extremely immunogenic (a dose of 0.158 MID₅₀ would seroconvert 50% of the monkeys) and that the dengue-3 vaccine was less than 20 fold attenuated with respect to the wild type virus.

Neutralization titers of all experimental animals used in this contract were obtained using duplicate samples. Most of the samples were assayed at least twice to confirm the results obtained.

c) Dengue-4 (strain 341750 Caribe) vaccine studies:

The next series of experiments were carried out to titrate a new candidate vaccine (Dengue-4 strain 341750 Caribe) and its parent virus. This work was initiated through an extension of our original contract. A group of 14 antibody free animals were brought to a mosquito-proof area. Seventeen antibody-free monkeys (from the previous experimental groups) were kept. A total of 33 dengue antibody-free monkeys were used in the new titration studies.

On February 17, 1987, groups of 5 animals were inoculated s.c. with 0.5 ml of different vaccine and virus doses. The vaccine doses used were: 1×10^6 MID₅₀, 5×10^4 MID₅₀ and 10^3 MID₅₀. The virus doses were: 1,900 MID₅₀, 190 MID₅₀ and 19 MID₅₀. The titers of vaccine and of virus on infection day were as follows.

Vaccine:	2×10^6 MID ₅₀ /ml	(2.6×10^5 pfu/ml)
Virus:	3.3×10^7 MID ₅₀ /ml	(5.1×10^6 pfu/ml)

The diluent used was EMEM, 0.25% HSA. Three monkeys were used as uninoculated controls.

Animals infected or vaccinated with the dengue-4 preparations were bled on post infection (vaccination) days 30 and 60. Serum samples were assayed for neutralization antibodies against the parent, wild type dengue-4 virus (strain 341750 Caribe).

Results obtained are shown on tables XII and XIII. Using the Reed and Huench procedure, the following MID₅₀ were obtained.

Dengue-4 vaccine (strain 341750 Carib): 2.37×10^5 MID₅₀

Dengue-4 virus (strain 341750 Carib): 60 MID₅₀

It is therefore concluded that the dengue-4 vaccine was highly attenuated. The MID₅₀ obtained for the parent dengue-4 virus agreed well with the previous result obtained with the H-241 seed of dengue-4 (see table VII).

TABLE I

Antibody Response of Monkeys Vaccinated with Different doses of
Candidate 45AZ-5 Dengue-1 Vaccine

Monkey Number	Vaccine Type	HID ₅₀	Antibody Titers on Post-Vaccination Day					
			30	60	90	120	150	190
D32	45AZ-5	1320	19	80	68	52	28	33
C43	45AZ-5	1320	18	26	140	14	<10	<10
C69	45AZ-5	1320	<10	10	80	18	<10	<10
B33	45AZ-5	1320	<10	50	210	14	<10	<10
E40	45AZ-5	1320	11	50	520	30	15	18
C49	45AZ-5	132	<10	<10	<10	<10	<10	<10
E09	45AZ-5	132	<10	<10	<10	<10	<10	<10
B24	45AZ-5	132	<10	<10	<10	<10	<10	<10
O27	45AZ-5	132	<10	<10	<10	<10	<10	<10
B91	45AZ-5	132	<10	<10	<10	<10	<10	<10
E36	45AZ-5	13.2	<10	<10	<10	<10	<10	<10
D56	45AZ-5	13.2	<10	<10	<10	<10	<10	<10
E11	45AZ-5	13.2	<10	<10	<10	<10	<10	<10
E24	45AZ-5	13.2	<10	<10	<10	<10	<10	<10
E32	45AZ-5	13.2	<10	<10	<10	<10	<10	<10

TABLE II

Antibody Response of Monkeys Vaccinated With Different Doses of
Candidate TP79-56 Dengue-1 Vaccine.

Monkey Number	Vaccine Type	ID ₅₀	Antibody Titers on Post Vaccination Day					
			30	60	90	120	150	190
F34	TP79-56	322	14	29	<10	<10	<10	<10
D41	TP79-56	322	<10	68	32	16	25	32
E22	TP79-56	322	<10	58	10	<10	<10	<10
E21	TP79-56	322	84	78	60	27	50	48
C67	TP79-56	322	<10	<10	<10	<10	<10	<10
E13	TP79-56	32.2	<10	<10	<10	<10	<10	<10
D42	TP79-56	32.2	<10	<10	<10	<10	<10	<10
E34	TP79-56	32.2	<10	<10	<10	<10	<10	<10
C89	TP79-56	32.2	<10	<10	<10	<10	<10	<10
E42	TP79-56	32.2	<10	<10	<10	<10	<10	<10
C73	TP79-56	3.2	<10	<10	<10	<10	<10	<10
C71	TP79-56	3.2	<10	<10	<10	<10	<10	<10
C42	TP79-56	3.2	<10	<10	<10	<10	<10	<10
C92	TP79-56	3.2	<10	<10	<10	<10	<10	<10
C46	TP79-56	3.2	<10	<10	<10	<10	<10	<10
B45	Controls							
D46	unvaccinated		<10	<10	<10	<10	<10	<10
D63								
C05								

TABLE III

Antibody Response of 45A2-5 Vaccinated Monkeys Challenged with
wild Type Dengue-1 Virus

Monkey Number	Antibody Titers on Post-Challenge Day		
	10	30	61
D32	31	30	36
C43	<10	41	96
C69	<10	42	62
B33	<10	58	90
E40	33	22	15
C49	<10	18	160
E09	<10	14	60
B24	<10	31	26
D27	<10	50	110
B91	<10	21	20
E36	<10	10	<10
D56	<10	110	160
E11	<10	42	100
E24	<10	84	60
E32	<10	10	74

Monkeys Were Challenged With 2916 MID50 of DEN-1 Virus (WP1974) on
Post Vaccination Day 190.

TABLE IV

Antibody Response of TP77-56 Vaccinated Monkeys Challenged With
Wild Type Dengue-1 Virus

Monkey Number	Antibody Titers on Post-Challenge Day		
	10	30	61
B36	<10	43	23
D41	19	35	88
B22	<10	20	80
B21	30	50	66
C67	<10	42	50
B13	<10	17	145
D42	<10	12	<10
B34	<10	17	82
C89	<10	140	310
B42	<10	13	<10
C73	<10	45	34
C71	<10	20	23
C42	<10	150	260
C92	<10	22	23
C46	<10	45	60
B45*	<10	30	105
D46**	<10	<10	<10
D63#	<10	20	56
C05*	<10	25	110

*Control, unvaccinated (but infected) monkey

**Control, uninfected and unvaccinated monkey

TABLE V

Antibody Response of Rhesus Monkeys Vaccinated With Different Doses of Candidate Dengue-3 (CH5349) Vaccine.

Monkey Number	Vaccine Dose (MID ₅₀ in 0.5 ml)	N Titers on	
		Post vaccination Day 30	60
G00	4,000*	45	N.D.
G57		22	N.D.
H13		28	N.D.
H16		27	N.D.
H19		10	N.D.
E07	400*	10	N.D.
E08		>640	N.D.
F86		>640	N.D.
G52		12	N.D.
G91		42	N.D.
F77	40*	170	60
F87		54	76
F89		240	58
G77		56	20
H11		>640	18

*Correspond to 1.21, 0.121 and 0.0121 pfu/0.5 ml, respectively.

N.D.= Not Done

TABLE VI

Antibody Response of Rhesus Monkeys Infected With Different Doses of Wild Type Dengue-3 (CH5349) Virus.

Monkey Number	Virus Dose ($10^{5.0}$ in 0.5 ml)	N Titers on Post Infection day	
		30	60
E44	15,000*	>640	N.D.
G49		500	N.D.
G75		>640	N.D.
H23		>640	N.D.
H24		520	N.D.
E23	1,500*	600	N.D.
E46		490	N.D.
G45		500	N.D.
H06		500	N.D.
H34		300	N.D.
G58	150*	300	N.D.
G78		>640	N.D.
G88		37	N.D.
G90		470	N.D.
H14		580	N.D.
G79	Unvaccinated,	<10	<10
H01	uninfected	<10	<10
H21	controls	<10	<10

*Correspond to 2.63, 0.263 and 0.0263 pfu/0.5 ml, respectively.

N.D.= Not Done

TABLE VII

Quantity of Dengue Virus Required to Infect Rhesus Monkeys

Vaccine or Virus	Dose in RID ₅₀	pfu	Animal Serocon- version	Type and Date of Report
DEN-2 (PR159)	10	9×10^{-2}	50%	Trans. R.S. Trop. Med. Hyg. 79, 248-251 (1985)
DEN-4 (H241)	22	1×10^{-2}	50%	Trans. R.S. Trop. Med. Hyg. 79, 248-251 (1985).
DEN-2/S-1 Vaccine	1.9×10^6	1.5×10^3	6/8	Final Report C-3029, June 25, 1984.
DEN-4 (H241 vaccine)	0.95×10^4	600	2/8	Final Report C-3029, June 25, 1984.
DEN-1 (45AZ-5 Vaccine)	417	1.1	50%	Quarterly Report C-4197, June 18, 1985.
DEN-1 (TP79-56 Vaccine)	116	0.7	50%	Quarterly report C-4197, June 18, 1985.
DEN-3 (CH53489)	0.158	0.9	50%	This report (table XI)
DEN-3 (CH3489 Vaccine)	2.93	6.9×10^{-4}	50%	This report (table X)
DEN-4 (341750 Caribe, Vaccine)	2.4×10^5	3×10^4	50%	This report (table XII)
DEN-4 (34150 Caribe)	60	0.77	50%	This report (table XIII)

TABLE VIII

Seroconversion of Rhesus Monkeys Inoculated With Varying Amounts of Dengue-3 Vaccine

Monkey Number	Vaccine Dose (MID ₅₀ in 0.5 ml)	Antibody titers on Post Vaccination Day	
		30	60
B348	12.5*	290	275
B594		420	336
B600		160	150
B860		410	320
E25		<10	<10
E26	3.125*	<10	<10
E29		98	100
E30		>80	84
E31		>89	84
E32		<10	<10

*Correspond to 29×10^4 pfu and 73×10^5 pfu, respectively

N.D.= Not done

TABLE IX

Seroconversion of Rhesus Monkeys Inoculated With Varying Amounts of Dengue-3 Virus (CH53489).

Monkey Number	Virus Dose (MID ₅₀ 0.5 ml)	Antibody Titers on Post Infection Day 30	60
E35	3.75*	100	110
E38		96	98
E39		160	145
E41		88	90
E46		93	107
E70	.375*	29	30
E71		<10	<10
E328		63	79
E329		48	60
E330		82	100
G79	Uninfected Controls	<10	<10
H01		<10	<10
H21		<10	<10

*Correspond to 21×10^{-4} pfu and 21×10^{-5} pfu, respectively.

N.D.= Not Done

Table X

Seroconversion of Rhesus Monkeys Inoculated With Varying Amounts of Dengue-3 Vaccine (CH53489)

Monkey Number	Vaccine Dose (MID ₅₀ in 0.5 ml)	Antibody titers on Post Vaccination Day	
		30	60
B348	12.5*	290	275
B594		420	336
B600		160	150
B860		410	320
E25		<10	<10
E26	3.125*	<10	<10
E29		98	100
E30		80	84
E31		89	84
E32		<10	<10
F13	0.3072*	<10	<10
G50		<10	<10
G84		<10	<10
G85		<10	<10
G94		<10	<10

*Correspond to 29×10^4 pfu, 73×10^5 pfu and 15×10^{-5} pfu, respectively

Table XI

Seroconversion of Rhesus Monkeys Inoculated With Varying Amounts of Dengue-3 Virus (CH53489).

Monkey Number	Virus Dose (MID ₅₀ 0.5 ml)	Antibody Titers on Post Infection Day	
		30	60
E35	3.75*	100	110
E38		96	98
E39		160	145
E41		88	90
E46		93	107
E70	.375*	29	30
E71		<10	<10
E328		63	79
E329		48	60
E330		82	100
G95	0.0297*	<10	<10
G96		<10	<10
G97		<10	<10
H12		<10	<10
H18		<10	<10
G79	Uninfected Controls	<10	<10
H01		<10	<10
H21		<10	<10

*Correspond to 21×10^4 pfu, 21×10^5 pfu and 79×10^5 pfu, respectively.

Table XII

Seroconversion of rhesus monkeys inoculated with different amounts of Dengue-4 Vaccine (strain 341750 Caribe)

Monkey number	Pfu 0.5 ml	MID ₅₀ 0.5 ml	D-4 Antibody Titers On Post vaccination Day	
			30	60
D24	1.3×10^5	1×10^6	40	41
E437			<10	38
F1			<10	35
G56			<10	50
G92			52	25
J1	6,500	50,000	<10	<10
J2			<10	<10
OH			<10	<10
E25			<10	50
E26			<10	<10
E32	130	1,000	<10	<10
F13			<10	<10
G50			<10	<10
G84			<10	37
G85			<10	<10

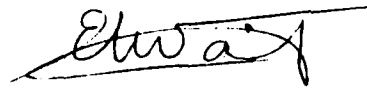
Table XIII

Seroconversion of rhesus monkeys inoculated with different amounts of wild type Dengue-4 virus (strain 341750 Caribe)

Monkey Number	Pfu 0.5 ml	MID ₅₀ 0.5 ml	D-4 Antibody Titers on Post vaccination Day	
			30	60
P1	25.5	1,900	66	190
P2			<10	170
P3			32	180
P4			<10	49
P5			47	230
P6	2.55	190	<10	<10
P7			<10	25
Winnie			<10	36
G94			<10	<10
E71			43	260
G95	0.255	19	<10	<10
G96			<10	43
G97			<10	<10
H12			<10	84
H18			<10	<10
G79			<10	<10
H01	uninfected		<10	<10
H21	controls		<10	<10

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," Prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978)



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