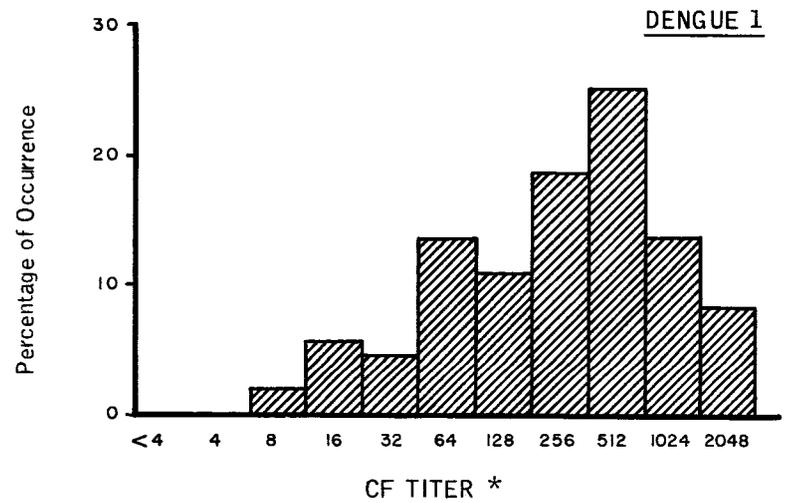
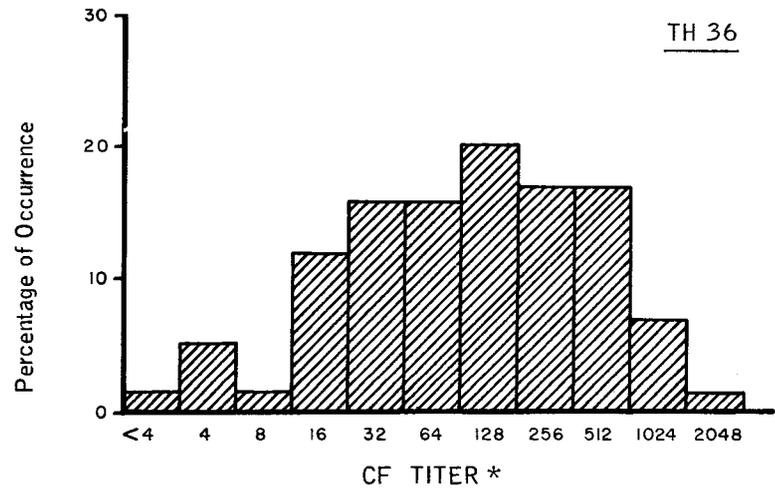
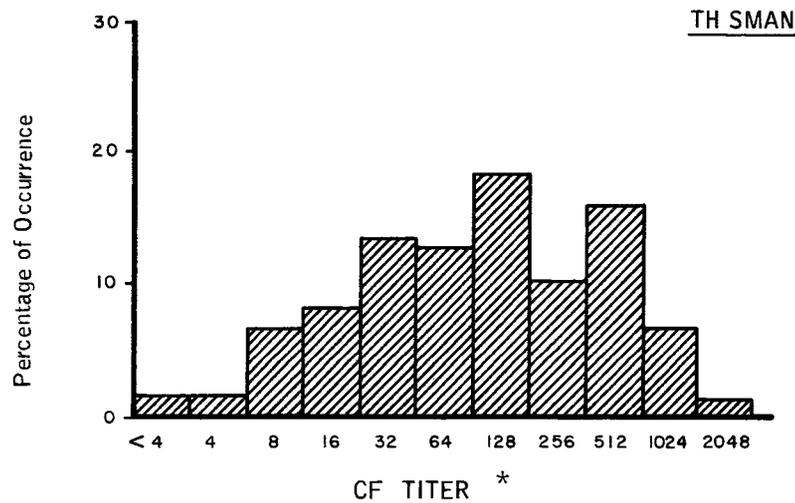
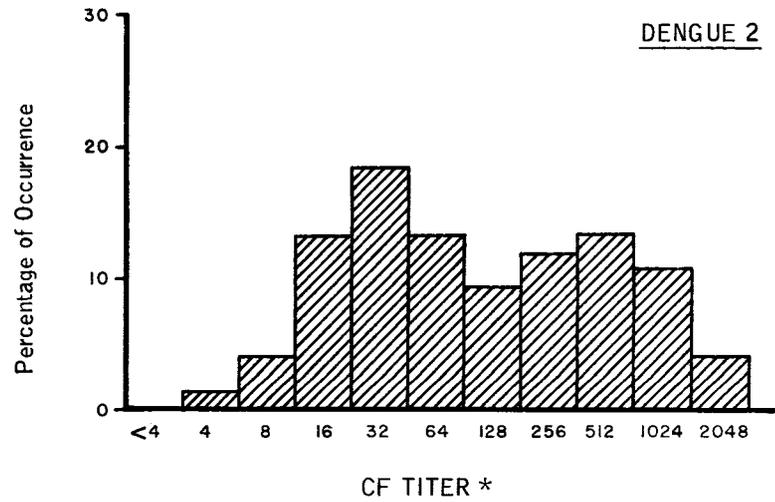




**FIGURE 1.** ARRAY OF TITERS IN 46 REPRESENTATIVE HEMORRHAGIC FEVER CONVALESCENT SERA COMPARING DENGUE 1 WITH TH SMAN CF ANTIGENS, BANGKOK PATIENTS, 1962



**FIGURE 2.** ARRAY OF TITERS IN 46 REPRESENTATIVE HEMORRHAGIC FEVER CONVALESCENT SERA COMPARING DENGUE 2 WITH TH 36 CF ANTIGENS, BANGKOK PATIENTS, 1962



\* EXPRESSED AS RECIPROCAL OF SERUM DILUTION

\* EXPRESSED AS RECIPROCAL OF SERUM DILUTION

residents of Thailand;

4. Antibody response in weanling mice to 2 or 3 dengue virus exposures. Acute and convalescent phase sera have collected from 4 groups of patients:

- a. Hospitalized hemorrhagic fever
- b. Patients hospitalized with febrile disease other than hemorrhagic fever
- c. Out-patients with miscellaneous febrile syndromes
- d. Surgical patients.

Acute phase plasmas were collected generally before the sixth day after onset of illness. Two weeks later a convalescent serum was obtained.

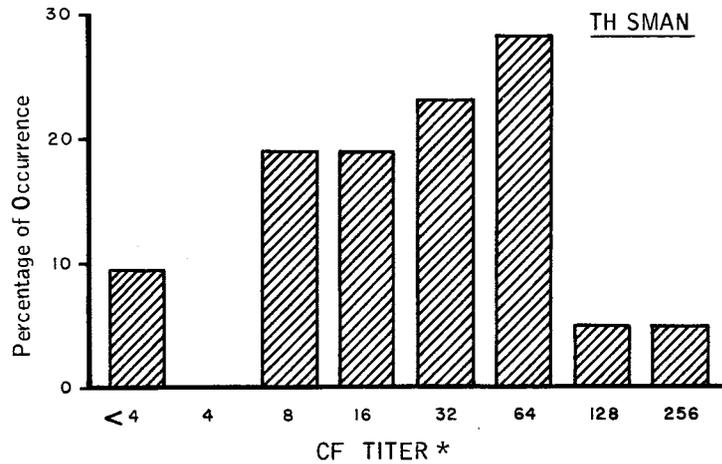
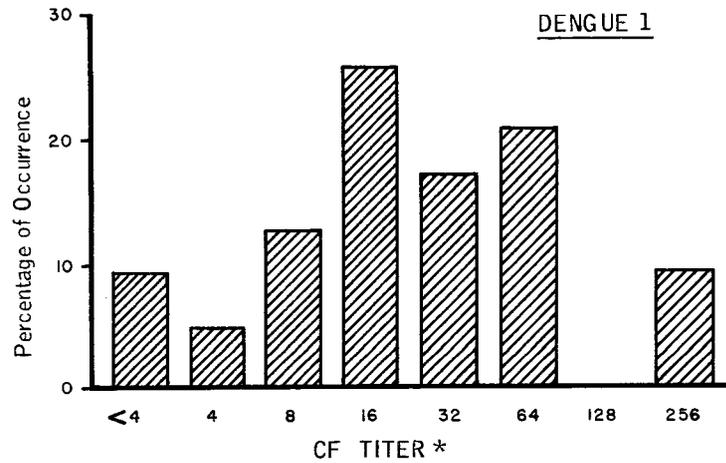
Acetone-ether extracted hemagglutination and complement-fixation antigens were used. All hemagglutination-inhibition tests were done in microvolumes using Microtiter equipment. The overnight hemagglutination-inhibition test as described by Clarke and Casals was employed. Sera were tested against 4-8 hemagglutination units. Known positive and negative human sera were used in all tests.

The complement-fixation (CF) test was done in microvolumes. Antigens and complement titrations were done in Kahn tubes. Two units of antigen and two exact units of complement and three units of anti-sheep hemolysin were used. Complement, serum and saline controls were maintained for each test as well as known positive sera which were constantly retested over a period of 2 years.

Progress: Selection of serologic antigens. Numerous studies have been undertaken to determine the number of distinctly different dengue virus antigens which could provide useful serologic information. In one such study, 46 hemorrhagic fever patients from whom 3 or more specimens were available were tested against high mouse passage prototype CF dengue 1-4 and TH-36 and TH-Sman. Figure 1 and 2 show that for this particular group of sera the correlation of antibody titers between dengue 1 and TH-Sman or between dengue 2 and TH-36 is good.

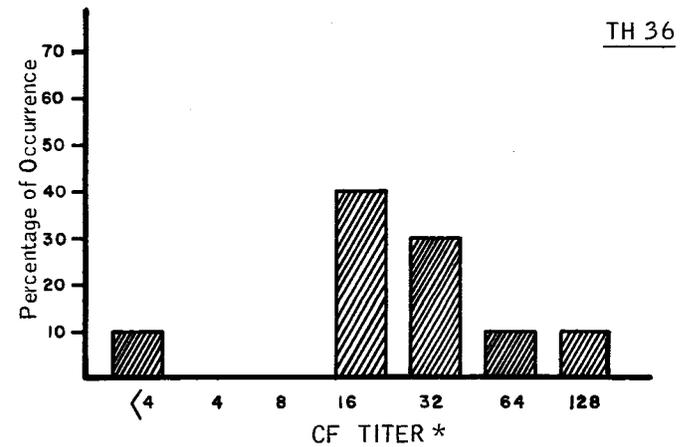
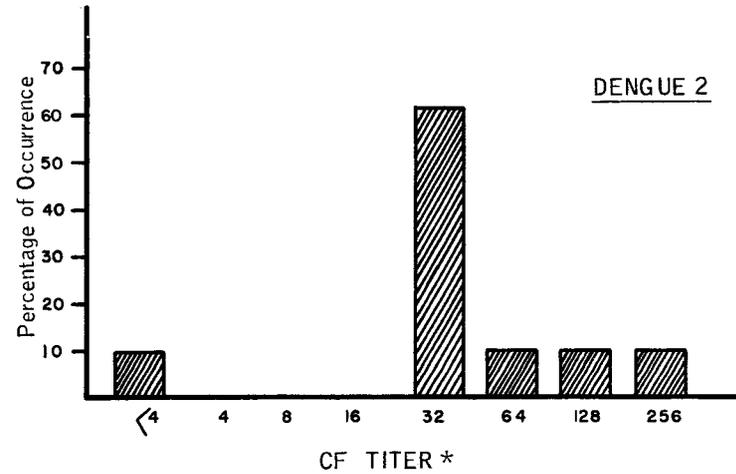
The antigenic similarity or dissimilarity of dengue 1 with TH-Sman and dengue 2 with TH-36 were studied further by examining convalescent sera from patients showing relatively specific CF antibody response (primary infection ?) who acquired hemorrhagic fever in small communities of Rayong and Ubol. In Rayong, eleven type 2 viruses were recovered from Aedes aegypti. In Ubol, twelve type 1 viruses were recovered from thirty four sera tested. Distribution of antibody in convalescent sera measured by type 1 and TH-Sman antigens or type 2 and TH-36 antigens are indicated in Figure 3 and 4. These studies have been extended to several hundred other human and animal sera, all with essentially the same result. In our experience results with dengue 1 or TH-Sman and dengue 2 or TH-36 did not

FIGURE 3. ARRAY OF TITERS IN 23 REPRESENTATIVE CONVALESCENT SERA FROM HEMORRHAGIC FEVER PATIENTS IN UBOL, THAILAND. DENGUE 1 (HAWAII) COMPARED WITH TH SMAN CF ANTIGENS.



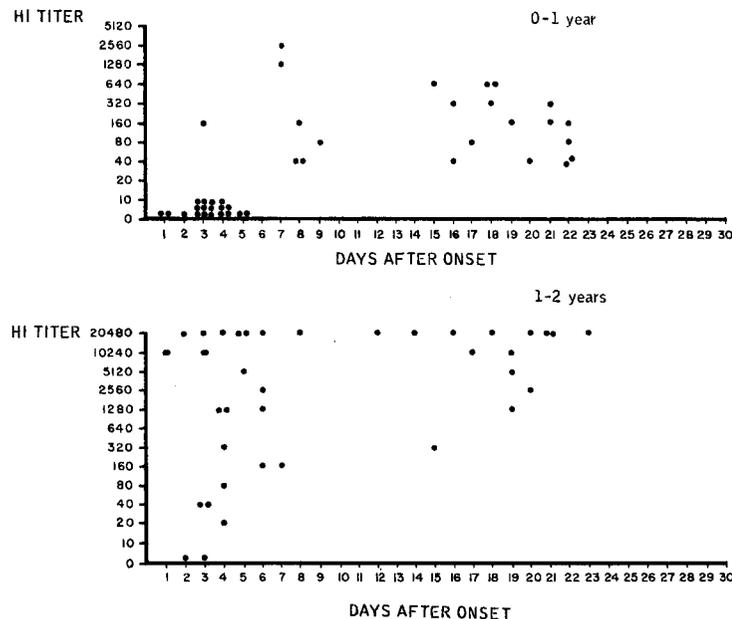
\* EXPRESSED AS RECIPROCAL OF SERUM DILUTION

FIGURE 4. ARRAY OF TITERS IN 10 REPRESENTATIVE CONVALESCENT SERA FROM HEMORRHAGIC FEVER PATIENTS IN RAYONG, THAILAND. DENGUE 2 (NEW GUINEA C) COMPARED WITH TH 36 ANTIGENS.



\* EXPRESSED AS RECIPROCAL OF SERUM DILUTION

FIGURE 5. SCATTER DIAGRAM OF HI ANTIBODY IN ACUTE AND CONVALESCENT SERA OF CHILDREN 0-1 AND 1-2 YEARS OLD WITH CLINICAL HEMORRHAGIC FEVER AND SEROLOGICALLY CONFIRMED DENGUE INFECTION.



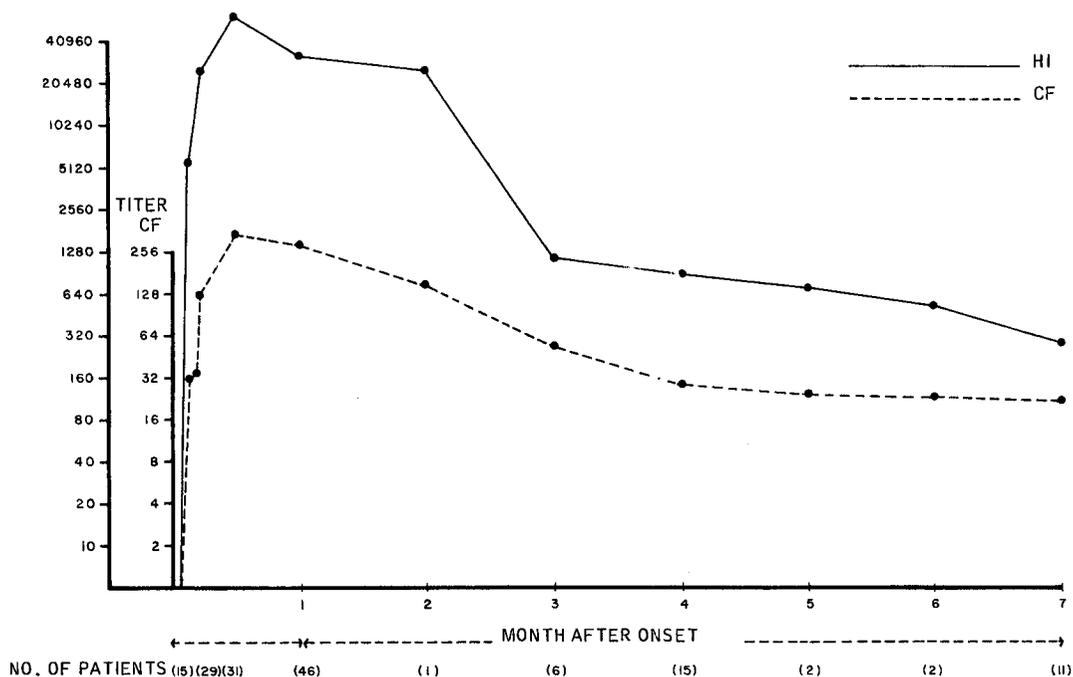
differ enough to warrant inclusion of the TH antigens in serologic tests .

HI and CF antibody response following dengue hemorrhagic fever in residents of Bangkok.

a. Multivalent HI and CF response. Forty six hemorrhagic fever patients with 3 or more serum specimens were selected for intensive study. The age and sex distribution of these patients resembles the distribution of hospitalized hemorrhagic fever except that children of less than 1 year were not included. HI titer results using d 1-4, TH-Sman, TH-36 and JE antigens were nearly the same; the antibody response to various CF antigens is predominantly multivalent. Antibody titers in convalescent serum to dengue 3 and 4 CF antigens were lower than dengue 1 and 2 titers. Only rarely did a monovalent CF antibody response occur from which it might be possible to predict the infecting dengue virus type. For the sera studied, it appears that use of dengue 1 antigen for either HI or CF resulted in a greater number of "positive" diagnoses of dengue infection than with use of other antigens. Whether this is because dengue 1 is more antibody avid or that a greater number of Bangkok patients had previous dengue 1 antigenic exposure is not known.

b. Antibody response and age. Paired sera have been obtained from 22 children under the age of 1 with a clinical diagnosis of Thai hemorrhagic fever. The HI antibody response in this group is contrasted with the response in 21 children between 1 and 2 years of age in Figure 5. The marked difference in response is unmistakable. The response of children under the age of one appears to resemble

FIGURE 6. GEOMETRIC MEAN TITERS OF HI AND CF ANTIBODY IN ACUTE AND CONVALESCENT SERA OBTAINED UP TO 7 MONTHS FROM 46 PATIENTS WITH CLINICAL HEMORRHAGIC FEVER BANGKOK, 1962



the antibody response of Caucasians, i. e., response to initial infection. Most acute phase sera are HI antibody negative and are followed by a rather low antibody response in most 1 year old children with hemorrhagic fever. Most antibody responses in children over 12 months appear to be of the secondary type. Antibody surveys of the Bangkok population in the same year showed that not more than 15% of children at 1-2 years of age have dengue HI antibody. If the observed antibody response of 1 year old children is the secondary type then preceding dengue infection in 1 year old children is considerably more frequent in HF cases than in the normals. This would suggest that hemorrhagic fever is a disease following secondary dengue exposure.

c. Development and duration of HI and CF antibody following dengue hemorrhagic fever.

Figure 6 shows the geometric mean titers of dengue HI and CF antibody in acute and convalescent sera obtained over a period of 7 months in 46 patients with hospitalized hemorrhagic fever. A very rapid development of HI and CF antibody characterizes the average patient's response to infection during the first 7-10 days of fever. Three months after infection, geometric mean HI titers have fallen to 1:768, while CF titers are at 1:35.2. These data appear to have some value for dating dengue infection in an average Bangkok resident. In this laboratory, HI titers of 1:640 or higher in convalescent sera of patients with a disease resembling Thai hemorrhagic fever are considered as presumptive evidence of recent dengue infection. The frequency of HI antibody titers of 1:640 or higher in 103 surgical patients without recent febrile illness studied during and after seasonal dengue virus dissemination in 1962 was 3.8%.

Table 1

COMPLEMENT-FIXATION TEST OF HYPERIMMUNE SERUM PREPARED  
TO ISOLATES (MULTIPLE STIMULI).

Bangkok virus hyperimmune serum	Reciprocal of CF antibody titer vs. 2 units of indicated prototype dengue antigen						
	D1	D2	D3	D4	TH-36	TH-Sman	Homologous virus
2358-62	4*	32	0**	4	4	4	64/128 ***
3149-62	16	8	8	4	4	16	16/32
5031-62	16	4	4	4	8	8	16/32
BKM 60-62	0	16	0	0	8	0	16/32
BKM 331-62	16	8	64	8	0	8	32/32
BKM 418-62	16	32	16	8	32	0	64/128

\* = Signifies reciprocal of highest dilution of serum which gives 2+ or more fixation of complement.

\*\* = No fixation at 1:4 dilution of serum.

\*\*\* = The numerator represents the maximum dilution of serum fixing complement in the presence of antigen and the denominator the maximum dilution of an antigen fixing complement in the presence of antiserum.

Table 2

COMPLEMENT-FIXATION TEST USING IMMUNE SERUM PREPARED  
BY TWO INOCULATIONS OF INDICATED VIRUSES

	Reciprocal of antibody titer vs. indicated 2 units of prototype dengue antigen					
	D1	D2	D3	D4	TH-36	TH-Sman
2358-62	0*	32	0	0	16	0
3149-62	16	0	0	0	0	8
5031-62	4	0	0	0	0	8
BKM 60-62	0	8	0	0	8	0
BKM 331-62	0	0	16	0	0	0
BKM 418-62	0	4	0	0	4	0

\* No fixation at a 1:4 serum dilution.

### Antibody response of weanling mice to dengue immunization.

Technique 1: Fresh 10% suspensions of mouse brain in saline were inoculated into 21 day old mice intraperitoneally (0.2 ml. amounts). After five injections at one week intervals, trial bleedings were made to determine whether satisfactory antibody titer had been obtained.

Technique 2: Three to four week old mice were inoculated IP with 0.3 ml of 10% fresh mouse brain saline suspension. Two weeks later mice were inoculated with 0.03 cc of  $10^{-2}$  of mouse brain suspension intracerebrally. (IC). A trial bleeding was performed ten days later. If it revealed no satisfactory titer, 0.03 cc of  $10^{-1}$  virus suspension was injected into mice intracerebrally. Trial bleeding was made 2 weeks later. Results of CF tests of antiserum prepared by technique 1 are shown in Table 1. Marked heterologous crossing is obvious. When fewer inoculations of serum were made, more specific results shown in Table 2 were obtained. Extensive studies of dengue mouse immune sera prepared to Bangkok dengue viruses have been made using dengue 1-TH-Sman and dengue 2-TH-36 antigens. Correlations are shown in Tables 3 and 4. Data suggest the antigenic homogeneity of the dengue 1-TH-Sman and dengue 2-TH-36 groups respectively.

Summary and Conclusions: Studies during the report period have shown that following HF infection caused by dengue viruses the HI antibody response is prompt and of great magnitude. The mean HI titer of 31 sera obtained 8-14 days after onset of fever was 1:40,960. Beginning at 1-3 months after infection HI antibody begins to decline and 7 months after infection mean values are below 1:320. CF antibody after dengue infection follows an almost identical pattern except that values are approximately 10-100 fold lower. Serologic response to chikungunya virus is quite different. HI antibody develops less rapidly than following dengue infection with average of 1:900 achieved 15-30 days after infection. Development of complement-fixation antibody is definitely delayed; most sera do not show any CF activity until 15-30 days after infection. Thus, sera having high HI antibody but low or no CF antibody to chikungunya suggest recent infection antibody titers in human serum measured with dengue 1 and 2 antigens were similar to titers obtained with TH-Sman and TH-36 antigens, respectively. By the HI test the infecting dengue virus could not be determined since antibody titers to all dengue types and Japanese encephalitis virus were nearly identical. In Bangkok area, CF results were only slightly more specific. The average patient developed CF antibody of about the same titer to 2 or more dengue viruses and Japanese encephalitis virus. Weanling mice inoculated IC and IP with 2 or 3 injections of low mouse passage dengue viruses developed low titered CF antibody which was dengue type specific. CF titers to dengue 1 and 2 antigens were the same as to TH-Sman and TH-36 antigens, respectively.

Table 3

CORRELATION TABLE SHOWING CF ANTIBODY TITERS OF 26 BANGKOK  
DENGUE MOUSE SERA TO DENGUE 1 (HAWAII) AND DENGUE TH-SMAN  
ANTIGENS

Reciprocal titer vs. dengue 1 (Hawaii) antigen

	Reciprocal CF titer	4	4	8	16	32
Reciprocal	4	1				
titer vs.	4		2	2		
TH-Sman	8		4	5	1	
antigen	16			2	4	1
	32			1	1	2

Table 4

CORRELATION TABLE SHOWING CF ANTIBODY TITERS IN 44 BANGKOK  
DENGUE VIRUS MOUSE IMMUNE SERA TO DENGUE 2 (NEW GUINEA C)  
AND TH-36.

Reciprocal titers vs. dengue 2 (New Guinea C) antigen

	Reciprocal CF titer	4	4	8	16	32
Reciprocal	4	1	2	3		
titer vs.	4		3	3	1	
TH-36	8	3	1	4	4	2
antigen	16			2	4	5
	32			1	1	3