

Isolation of Dengue Viruses from *Aedes aegypti*  
Collected in Bangkok, Thailand

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**OBJECTIVE :** To isolate dengue viruses from *Ae. aegypti* for studies of the biological and antigenic properties of these viruses.

**BACKGROUND :** Studies in progress to characterize antigenic and biological properties of wild dengue viruses required field collections of strains of these viruses. According to previous findings, dengue viruses can be isolated from *Aedes aegypti* collected in and around homes of dengue hemorrhagic fever (DHF) patients (1). The isolation rate observed during investigations conducted at Koh Samui, Thailand during 1968 was 1 isolate for every 19 female *Ae. aegypti* assayed. Similar attempts were made during 1976 to isolate dengue viruses from homes of DHF patients in Bangkok; however, the isolation rate was only one dengue virus isolation from over 200 *Ae. aegypti* collected from homes.

**MATERIALS AND METHODS :** Adults and immature *Aedes aegypti* and *Culex* species mosquitoes were collected during the latter month of 1978 and during 1979 in the homes of DHF patients in Bangkok. Male and immature *Ae. aegypti* were collected for dengue virus transovarial transmission studies described in another section of this Annual Progress Report. The mosquito collecting team included the principal investigator, one entomology technician and a nurse. The collecting period was approximately one hour for each house and all mosquito collections were made between 1330 and 1530 hours. Individual female *Ae. aegypti* mosquitoes were captured in 2 x 8 cm vials while attempting to feed on members of the collecting team. *Culex* species were captured while resting on the walls primarily of bathrooms in vials similar to that used for *Ae. aegypti*.

Mosquitoes were transported to the laboratory where they were identified and subsequently processed for virus isolation studies. Engorged mosquitoes were retained in individual vials until eggs were laid. A 5% sucrose solution was provided to mosquitoes continuously. Individual mosquitoes were triturated in a sterile tissue grinder in the presence of 1.0 ml of RPMI 1640 medium that contained 10% fetal calf serum (FCS), 500 units of penicillin/ml and 500 ug of streptomycin/ml. The pH of the medium was adjusted to 8.2-8.4 by adding 7.5% NaHCO<sub>3</sub>. Mosquito suspensions were centrifuged for 30 minutes at 4°C at

1000 rpm. The supernatant of each suspension (0.3 ml) was inoculated onto confluent monolayers of LLC-Mk2 cells, two cultures per specimen, one for direct and one for delayed plaque assay. Cell cultures were maintained at 35°C. Some suspensions were also assayed for virus by intrathoracic inoculation of *Toxorhynchites splendens* that were maintained at 32°C.

Virus isolates were identified by plaque reduction neutralization tests employing dengue virus specific antiserum. Stock viruses of each isolate, using original mosquito suspensions were prepared in *Ae. aegypti* and in *Tx. splendens* and if possible, in LLC-Mk2 cells

RESULTS : *Ae. aegypti* were collected from a total of 24 houses during September 1978 through July 1979 (Table 1). Of the 24 houses, the patients from 22 were proven dengue virus infection. Collections were made on the average of 6.5 days (range 1-21 days) after patients were admitted to the Children's Hospital. Dengue viruses were isolated from *Ae. aegypti* collection in 4 of the 22 houses or 1 virus isolated per 5.5 houses. The results of virus isolation attempts from *Ae. aegypti* are presented in Table 1. A total of seven dengue virus type-2 isolates were made from 168 individually assayed mosquitoes. Multiple isolations were obtained from 3 of the 4 houses that yielded infected mosquitoes. Also, dengue-2 virus was isolated from patients who resided in 2 of the 4 houses. A summary of information concerning virus isolations is presented in Table 2. All mosquitoes were assayed by direct and delayed plaque assay in LLC-Mk2 cells, while 71 of the 157 were assayed in *Tx. splendens*. The same dengue viruses that were isolated in LLC-Mk2 cells were also isolated in *Tx. splendens*. Each of the seven isolates were reisolated from the original mosquito suspensions.

Stock viruses of isolates were prepared in *Ae. aegypti*, *Tx. splendens* and when possible in LLC-Mk2 cells employing the original mosquitoes as inoculum. A list of stock viruses and history of each stock is presented in Table 3.

#### REFERENCES :

1. Russell, P.K., Yuill, T.M., Nisalak, A., Udomsakdi, S., Gould, D.J., and Winter, P.E., 1968. An Insular Outbreak of Dengue Hemorrhagic Fever. II Virologic and Immunologic Studies Am. J. Trop. Med. and Hyg. 17: 600-608.
2. Scott, R.M., Gould, D.J., and Nisalak, A. Dengue Virus Isolation from Patients and Mosquitoes. Annual Progress Report, AFRIMS, pp. 82-83, 1976-1977.

Table 1. Summary of data concerning the isolation of Dengue Viruses from *Aedes aegypti*, Bangkok, Thailand, 1978-79.

Collection Date	Number of Collections	Number of Mosquitoes	No. Positive/ No. tested
Sept 78	2*	16	2/16
Oct 78	2	36	0/36
Jan 79	4	22	0/22
Feb 79	1	03	2/03
Mar 79	2	07	2/07
Apr 79	3	04	0/04
May 79	2	13	0/13
Jun 79	3	42	0/42
Jul 79	3	25	1/25
	22	168	7/168(1:22.4)**

\* Number of houses surveyed for mosquitoes.

\*\* One *Ae. aegypti* per 22.4 mosquitoes tested yielded a dengue virus isolation.

Table 2. Dengue viruses isolated from *Aedes aegypti* collected in homes of dengue hemorrhagic fever patients in Bangkok, Thailand, 1978-1979.

Date Mosquitoes Collected	Houses Number	Cases Number	No. Mosq. Collected	No. Positive/ No. tested	Virus Identity
20 Sept 78	189	D78-091	14	2/14	Den-2
28 Feb 79	008	D79-016	02	2/2	Den-2
16 Mar 79	434/8	D79-024	08	2/8	Den-2
19 Jul 79	35/42	D79-076	18	1/18	Den-2

Table 3. Infectivity titers of reference stocks of dengue virus type-2 strains in LLC-Mk2 cells and in *Toxorhynchites splendens*.

Virus Stock	Date Isolated	Source	Passage History	Infectivity Titers LLC-Mk2 Cells
Den-2 (78-091A)	20 Sept 78	<i>Ae. aegypti</i>	Ae-2* Ae-1, TS-1**	ND 2.5 x 10 <sup>5</sup>
Den-2 (78-091B)	20 Sept 78		Ae-2 Ae-1, TS-1	ND 1.0 x 10 <sup>5</sup>
Den-2 (79-016-1F)	28 Feb 79		Ae-2 Ae-1, TS-1	3.0 x 10 <sup>4</sup> 20 x 10 <sup>4</sup>
Den-2 (79-016-2F)	28 Feb 79		Ae-2 Ae-1, TS-1	7.0 x 10 <sup>4</sup> 2.0 x 10 <sup>4</sup>
Den-2 (79-024-11F)	16 Mar 79		Ae-2 Ae-1, TS-1	1.0 x 10 <sup>4</sup> 3.0 x 10 <sup>4</sup>
Den-2 (79-024-13F)	16 Mar 79		Ae-2 Ae-1, TS-1	5.0 x 10 <sup>2</sup> 3.0 x 10 <sup>3</sup>
Den-2 (79-076-1F)	19 Jul 79		Ae-2 Ae-1, TS-1	ND

\* Ae-2 indicates that the virus had received one passage in *Ae. aegypti* after original isolation from *Ae. aegypti*.

\*\* Ae-1, TS-1 indicate that the virus had received one passage in *T. splendens* after original isolation from *Ae. aegypti*.

\*\*\* Plaque forming units/0.3 ml.