

## Isolation of Dengue Virus from Patients in Provincial Hospitals

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**OBJECTIVE :** To isolate dengue viruses from patients seen at provincial hospitals and compare the virus serotypes with those isolated in Bangkok.

**BACKGROUND :** Since 1962 the Medical Research Laboratory has isolated dengue viruses from patients at the Children's Hospital in Bangkok. Through this period several shifts in the dengue types occurring in the city have been documented. Dengue 2 virus has been present in Bangkok each year that isolations were attempted. In the early 1960's Dengue 1, 3 and 4 were also present. Dengue 4 was isolated in 1965 but from 1969 through 1975 it was not found in Bangkok patients. From 1972 through 1975, dengue 1 and 3 made up approximately 50% of the isolates with dengue 2 making up the rest. In 1976 dengue 1 and 3 appeared to have been completely replaced by dengue 4.

During 1977 the incidence of dengue infections throughout Thailand increased earlier than expected and to unprecedented levels. Possibly this increase represents the reintroduction of Dengue 4 throughout the country.

Study of the history of dengue infection in Thailand suggests that virus transmission has been endemic in Bangkok since the late 1950's if not before. Throughout the years sporadic outbreaks of dengue occurred in the provincial areas, possibly representing introduction of dengue virus into these areas from Bangkok. Over the past several years dengue infection has been occurring regularly in certain provincial centers; e.g., Ubol, Udorn, Pitsanuloke, Khon Khean, and Chanthaburi. It has been suggested that in these areas dengue may have become endemic. The Laboratory has not attempted isolation from upcountry provincial areas for many years. The purpose of this study was to isolate dengue virus from patients seen in provincial hospitals and to compare the virus serotypes to those isolated in Bangkok.

**METHODS :** Following consultation with Dr. Sujarti, collection teams were placed in provincial hospitals found to be admitting large numbers of patients with clinical dengue hemorrhagic fever (DHF). These teams, with the help of the

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director and the pediatric staff of the hospital, collected bloods on patients with signs and symptoms of dengue infections. Acute serum and heparinized plasma were obtained on patients who had been clinically ill for four days or less. A convalescent serum was then collected two to five days later. A short history and physical examination was recorded on each patient.

Serum and plasma samples were stored in liquid nitrogen and transported to the laboratory. Routine virus isolation was carried out on acute plasma using a direct and delayed plaque technique on LLC-Mk<sub>2</sub> cells. Viruses were identified by a plaque reduction neutralization technique using monkey antisera made to prototype dengue virus serotypes.

RESULTS : At the time of this report, 35 viruses have been isolated from specimens collected from four provincial medical centers (Table 1). Identification of the virus isolated, serology on the patients collected and integration of this data with the historical and demographic information collected will be under taken.

Table 1. Virus Isolations from Patients Admitted to Provincial Medical Centers in Thailand.

Provincial Hospital	Number Studied	Isolation	
		No.	%
Phrae	23	7	(30.4)
Khon Kaen	42	14	(33.3)
Ubon	34	4	(11.8)
Udorn	24	10	(41.7)
<b>Total</b>	<b>123</b>	<b>35</b>	<b>(28.5)</b>