

ISOLATION OF FLAVIVIRUSES FROM BLOOD SPECIMENS FROM
PALM OIL AND RUBBER PLANTATION WORKERS IN
CENTRAL MALAYSIA (CONTINUED)

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OBJECTIVES, BACKGROUND AND METHODS : See AFRIMS Annual Progress Report, October 1979 to September 1979, pp. 239-247.

RESULTS : In the last annual report it was noted that 21 virus isolates were obtained from the 84 acute frozen whole blood specimens assayed by seven methods. Since that time the coded HAI sero responses of the individual patients have been forwarded to AFRIMS and the isolation results have been analyzed with regard to the HAI sero-responses of the patients; these results are presented here. Although in the last annual report it was stated that all patients had an 8 fold or greater HAI titer rise against a representative Flavivirus antigen (Tembusu) and that the frozen blood specimens were obtained within 7 days of onset, those criteria were not rigidly adhered to in the selection of specimens for isolation attempts. Table 1 shows that in fact only 28 patients of the patients from whom isolations were attempted had a definite 8 fold or greater titer rise. In addition 28 patients had a high fixed titer of at least 1:1280 in either the acute or convalescent specimen, 12 had a four fold titer rise, and 4 had less than a four fold titer rise. Among the 28 patients with rigorous proof of sero-conversion (i.e., an eight fold rise) the isolation rate was 64% (18/28); of the remaining 52 specimens with less rigorous serologic proof of infection (high fixed titer or four-fold rise) the isolation rate was 6% (3/52). As the isolation success rates were so strongly a function of the serologic diagnostic criteria used, we therefore chose only to analyze those cases in which conventional criteria were met, i.e., a four-fold or greater serologic rise and/or a titer of 1:1280 or greater in either the acute or convalescent specimens. For each of the four main techniques used an equation was derived by multiple linear regression analysis to determine the probability of a positive isolate as a function of both the acute serum HAI antibody titer and the acute serum day of illness where

X = day of illness (onset = day 1)

Y = $\log_2 \frac{(\text{HAI titer})}{10}$ (if HAI titer < 20 Y = 0
20 Y = 1
20 Y = 2
etc.)

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Z = probability of a positive isolate

So that

$$Z_n = A_0 - A_1 X_n - A_2 Y_n$$

The results are presented in Table 2. For all four techniques, the day of illness was found to have little impact on the isolation success rate, while the antibody titer had a strong influence.

Among those 68 patients on whom this analysis was performed almost all showed a secondary type flavivirus antibody response (antibody present in acute serum in 62 cases; convalescent titer ≥ 1280 in 60 cases).

In this population of Malaysians showing a sero-conversion to a representative Flavivirus antigen the approximate "idealized" isolation rates (calculated isolation rate on day 1 with HAI titer < 20) were as follows :

Mosquito inoculation	75%
Suckling mouse inoculation	67%
LLC cells	38%
C6/36 cells	14%

The possibility remains, untested, that these isolation rates are falsely low due to the presence of an undetected non-dengue flavivirus in some of the patients.

Table 1. Isolation results in patients meeting various serologic criteria for flāvivirus infection

<u>Serologic criteria met*</u>	<u>Isolation results</u>	
	(+)	(-)
All <u>$\geq 4x$</u>	19	21
4 x	1	11
<u>$\geq 8 x$</u>	18	10
All 640	2	38
640	1	11
<u>≥ 1280</u>	<u>1</u>	<u>27</u>
Total	21	59

* 4 cases excluded as not meeting absolute minimal criterion of a 4 fold titer rise.

Table 2. Comparison of multiple linear regression analyses of different isolation techniques

$$Z = A_0 + A_1 (\text{day of illness}) + A_2 \log_2 \frac{(\text{Acute HAI titer})}{10}$$

Methods	Constants			Calculated probability of an isolate on day 5 with an HAI titer of 1:320
	A0	A1	A2	
Mosquito inoculation	.76	-.010	+.080	31%
Suckling mouse inoculation with virulent virus challenge	.70	-.033	-.056	26%
LLC cells	.39	-.013	-.038	13%
C6/36 cells	.14	+.001	-.016	6%