

## The Sensitive Host for Recovery of Japanese Encephalitis Virus (JEV)

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**BACKGROUND:** In the previous Annual Report, Aedes albopictus cells (Singh line) were shown to be equally or more sensitive than LLC-MK2 cells for the growth of arboviruses recovered in Thailand. The viruses tested were the 4 serotypes of dengue, JEV, Wesselsbron, Ingwavuma and Tembusu viruses. The virus titer obtained in Aedes albopictus cells was generally higher than that in MK2 cells and the peak virus titer in A. albopictus cells appeared earlier than in MK2 cells. The present study was designed to compare A. albopictus cell and MK-2 cells as isolation systems for recovery of JEV from the brain of fatal human cases and from the brain of primates intracerebrally inoculated with various strains of JEV.

**METHODS:** The host systems used for virus isolation were A. albopictus cells, LLC-MK2 cells and one-day old mice. The method of virus assay was plaque titration in LLC-MK2 cells.

Before inoculation, brain suspensions were serially diluted ten-fold and each dilution was inoculated into A. albopictus cells, MK2 cells and one-day old mice. The sensitivity of the 2 tissue cultures was defined as the highest dilution of brain suspension that yielded a virus titer of not less than  $10^{2.0}$  in the fluid phase of the infected cell culture. In tissue culture systems half of the fluid supernatant was sampled and replaced with equal volumes of fresh medium. The period of study was 7 days. In mice, the morbidity ratio of the first brain passage was used to indicate the growth of virus. Two litters (16 mice) were used for the intracerebral inoculation of each brain dilution. The volumes of virus inoculation in tissue culture and mice were 0.3 ml and 0.02 ml respectively.

**PROGRESS:** 1. Recovery of JEV from experimental monkeys: Five monkeys were inoculated intracerebrally with  $7 \times 10^{4.0}$  to  $1 \times 10^{7.0}$ . PFU JEV per 0.25-0.5 ml inoculum. The passage history of the virus used in each monkey is shown in Table 1; two JEV strains were isolated from infected mosquito pools while the third strain was originally isolated from a human brain. Within 7 days after inoculation the monkeys developed intermittent fever, lethargy, paresis and collapse. The monkeys were sacrificed within 1 or 2 days after symptoms developed, the brains removed, and portions frozen for virus recovery and fixed for histopathological analysis.

Monkey y-0102 was not killed 1 to 2 days after paresis developed but rather 2 weeks later. It was anticipated that the virus titer in the brain of this monkey would be lower than the 4 other subjects and that the low titer would better test the abilities of the isolation systems to recover JEV.

The results in Table 1 show that A. albopictus cells are more sensitive than MK2 in the recovery of JEV from monkey brains, and generally detected virus at a 10-fold less dilution than obtained in MK2 cells. No virus was recovered in either cell culture in the monkey killed after 2 weeks of paralysis. Both cell cultures were more sensitive than suckling mice in these experiments.

2. Recovery of JEV from human brain: Suspensions of human brain from encephalitis cases were used to test the recovery of JEV by direct and delayed plaque in LLC-MK2 cells and in suckling mice; the results are shown in the last two columns of Table 2. Remaining suspensions stored at  $-70^{\circ}\text{C}$  were thawed, diluted ten-fold and inoculated into A. albopictus cells, MK2 cells and one-day old mice. Agreement between initial isolation and reisolation results in MK2 cells was found. In the reisolation, A. albopictus cells were more sensitive than MK2 cells except for specimen 48642. A. albopictus cell line

is the only host that recovered JEV from brain specimen 45342 from which JEV was not isolated initially. The mouse isolation technique failed to demonstrate JEV growth of 48197 (reisolation) and 48642 (primary isolation).

These results would suggest that A. albopictus cell line may be more sensitive than MK2 for isolation of JEV from infected brain. The data would gain added significance if replicate experiments were performed in order to confirm the initial promising results. Suckling mice in these experiments were far less sensitive than both tissue cultures.

Table 1.  
The Sensitivity of A. albopictus Cells, LLC-MK2 Cells and One-day Old Mice  
in the Recovery of JEV from Experimental Monkey Brains

Monkey number	JEV strains	Log <sub>10</sub> dilution of brain yielding 10 <sup>2.0</sup> virus titer on day 7		Log <sub>10</sub> dilution of brain causing sickness in one-day old mice	Mouse morbidity ratio: mice sick / mice tested
		LLC-MK2	<u>A. albopictus</u>		
y-090	BKM1096,MK2(1)	1	2	negative	0/16*
y-091	BKM1096,MK2(1)	4	5	undiluted 1	16/16 2/16
y-097	BKM1022,MK2(1)	5	6	3 4	16/16 3/16
y-099	40783,MK2(1)	6	6	4 5	16/16 4/16
y-0101	40783,MK2(1)	3	3	undiluted	2/16
y-0102	40783	This monkey was killed after two weeks of paralysis. No virus was detected in cell cultures and mice showed no symptoms after inoculation			

\* Undiluted brain

Table 2.  
The Sensitivity of A. albopictus Cells, LLC-MK2 Cells and One-day Old Mice  
In the Recovery of JEV from Human Brain.

Specimen number	Log <sub>10</sub> dilution of brain yielding ≥ 10 <sup>2.0</sup> virus titer on day 7		Results of mouse inoculation (morbidity ratio)	Result of primary isolation**	
	LLC-MK2	A. albopictus		LLC-MK2	Mice
45342 1970*	Negative	Undiluted	Negative	Negative	Negative
48197 1971	2.0	3.0	Negative	Positive	Positive
48642 1971	3.0	3.0	undiluted 1/16 10 <sup>1.0</sup> 0/16	Positive	Negative
48944 1971	1.0	2.0	Not done	Positive	Positive
49349 1971	1.0	2.0	Not done	Positive	Positive
48633 1971	Negative	Negative	Negative	Negative	Negative
48579 1971	Negative	Negative	Negative	Negative	Negative
48530 1971	Negative	Negative	Negative	Negative	Negative

\* Year of virus isolation

\*\* Inoculation with undiluted material