

## JE Virus Recovery after High Speed Centrifugation of Presumably Infected Mosquito Pools

Principal Investigators:            Debhanom Muangman, M.D., Dr. P.H.  
   Robert Edelman, LTC, MC  
   Douglas J. Gould, Ph.D.

**OBJECTIVE:** To determine 1) whether high speed centrifugation  
   or 2) the presence of large number of uninfected mosquitoes in a presumably  
infected mosquito pool would influence virus recovery from the supernatant fluid of that triturated pool.

**BACKGROUND:** In the Chiangmai JEV project, virus was isolated from triturated pools of various mosquito species clarified by high speed centrifugation (10,000 rpm at 4°C for 30 min). No experimental data are available which show the possible deleterious effect of such centrifugation on virus recovery. Furthermore Yuill (SEATO Lab) had reported in the past that the presence of large numbers of uninfected mosquitoes in a pool might decrease the chance of virus recovery in LLC-MK2 cell culture due to virus inhibitors released by mosquito tissues. The current study was designed to elucidate the effect of centrifugation on virus recovery and to confirm that mosquito tissues are indeed inhibitory in tissue culture.

**PROGRESS:** Presumably JEV infected Culex tritaeniorhynchus (13 days post infectious blood meal) were triturated with 99 uninfected Culex trl. in 4 ml. of Bovine Albumin Phosphate Saline (BAPS) which contained penicillin, streptomycin, and Fungicidin. A total of 10 such pools were triturated. The triturated mosquito pool was divided into 2 equal portions. The first portion was centrifuged at 10,000 rpm. at 4°C for 30 min. The second portion was left in a container surrounded by wet ice in a refrigerator at 4°C for the same period of time. After that, the two supernatant fluids were collected and tested for the presence of virus in suckling mice (I.C.) and in LLC-MK2 cell culture (direct and delayed plaque method). The results are shown in Table 1.

The results demonstrate no significant difference in virus recovery in centrifuged and non-centrifuged portions of each of the 10 mosquito pools. Although MK2 cell culture and suckling mice seem to be almost equally sensitive in detecting virus, contamination (especially with fungi) of tissue culture occurs about 5 times more frequently in uncentrifuged than centrifuged specimens. The suckling mice were apparently not affected by this contamination. Thus centrifugation apparently does not impair recovery of JEV from infected mosquito suspensions. On the same day of the previous experiment, 23 presumably JEV infected mosquitoes from the same infected mosquito lot were individually triturated in 1 ml. of BAPS, centrifuged, and tested for virus in MK2 cells. The results were compared with MK2 cell virus recovery from the 10 mosquito pools composed of 1 infected mosquito and 99 uninfected ones (Table 1). The results in Table 2 provide evidence that the presence of at least 99 uninfected mosquitoes in a pool of 100 mosquitoes did not interfere with the detection of virus in that pool as isolated in MK2 cell. Thus, we were unable to confirm Yuill's observation that large amounts of triturated mosquito tissues might have virus inhibitory effect in tissue culture.

Table 1.  
Virus Recovery from Centrifuged and Uncentrifuged Mosquito Suspensions in Suckling Mice and LLC-MK2 cell culture.

<u>Centrifuged</u>		<u>Uncentrifuged</u>	
<u>Suckling Mice</u>	<u>MK2</u>	<u>Suckling Mice</u>	<u>MK2</u>
2/10*	3/10	2/10	2/10

\*  $\frac{\text{No. pools positive}}{\text{No. pools tested}}$

Table 2.  
Infection Rates of Presumably Infected Culex tritaeniorhynchus Triturated alone (A) or with 99 Uninfected Mosquitoes of the Same Species (B)

	<u>Mosquito Pools—A*</u>	<u>Mosquito Pools—B**</u>
<u>No. Pools infected***</u>	6/23	3/10
No. Pools tested		
Infection rate	26%	30%

\* 1 Presumably — JE infected mosquito in 1 ml diluent

\*\* 1 Presumably — JE infected mosquito and 99 uninfected mosquitoes in 4 ml diluent

\*\*\* JE virus isolations in MK2 cell culture