

## USE OF CONCAVALIN A TREATED PS CELL CULTURES FOR THE DETECTION AND ASSAY OF JAPANESE ENCEPHALITIS VIRUS FROM MOSQUITOES

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The use of PS cell line for the growth and assay of JE virus has been recommended by several workers (1, 3). Concanavalin A (Con A) has been shown to enhance growth of JE virus in PS cells (2). Hence the sensitivity of Con A-treated PS cells for the assay of JE virus was tested in comparison with normal untreated PS cells and newborn mice. Four to five-day-old laboratory reared *Culex bitaeniorhynchus* mosquitoes were experimentally infected with JE virus (strain No. 724038) by oral route either by membrane feeding technique or by feeding upon viraemic chicks. Altogether 141 mosquitoes were tested employing tissue culture and suckling mice.

Individual mosquitoes were ground in 1.5 ml of phosphate saline with 0.75% bovine albumin, pH 7.2, containing 1000 units penicillin and 2 mg/ml of streptomycin and centrifuged at 10 000 rpm for one hour. The supernatant fluid was used as the inoculum for PS cell cultures — both Con A-treated and untreated — prepared in Laxbro plates and for intracerebral inoculation of 2-day-old mice, simultaneously. Plaques were counted wherever present. In the absence of plaques, cytopathic effect (CPE) was graded as ++ to +++++. Mice were observed for 20 days.

Sr. No.	Specimen No.	PS cell culture		Infant mouse system	
		Con A-treated	Untreated	MR	AST
1.	826400-34	2 Pl/W	N	0/8	20.0
2.	826400-36	2 Pl/W	N	0/8	20.0
3.	826400-70	8 Pl/W	N	0/8	20.0
4.	826400-71	+	6 Pl/W	1/8	18.4
5.	826783-22	+	N	0/8	20.0
6.	83624	2 Pl/W	N	1/8	18.4
7.	83424-1	6 Pl/W	1 Pl/W	1/8	18.4
8.	83424-3	2 Pl/W	3 Pl/W	0/8	20.0
9.	83424-12	10 Pl/W	4 Pl/W	7/8	8.1
10.	83424-17	10 Pl/W	7 Pl/W	5/8	11.5

MR — Mortality ratio; + — Cytopathic effect; Pl/W — Plaques per well;

AST — Average survival time; N — Not detected

Out of the 141 mosquito suspensions, 43 were found to be positive in all 3 systems. However in Con A-treated cells, 5 additional specimens were found positive which were negative in the other two assays. The Con A-treated PS cells always showed higher degree of CPE or higher number of plaques than the untreated ones. When inoculated with mosquito suspension of high virus titre, all mice died within 3 days. However, when the virus titre in the mosquito suspension was low, the mice showed an increased average survival time (AST) either due to late or fewer deaths, where as Con A-treated PS cultures showed clear CPE or plaques as indicated in the Table. JE virus titre of 15 individual mosquitoes was estimated using Con A-treated and untreated PS cell cultures. The results indicate that Con A-treated PS cultures showed significantly higher titres (G. M. 4.156) than the untreated PS cells (G. M. 3.279) ( $P < 0.05$ ).

## References

1. Inoue, Y. K., Ogura, R., *Virology* **16**: 205, 1962.
2. Kelkar, S. D., *Indian J. med. Res.* **81**: 437, 1985.
3. Kożuch, O., Mayer, V., *Acta virol.* **19**: 498, 1975.