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NAWARAT CHAROENSRI : PRODUCTION OF JAPANESE ENCEPHALITIS VIRUS IN BHK-21 CELLS USING LOW CHARGED DEAE-DEXTRAN MICROCARRIER AND SUSPENSION CULTURE SYSTEMS. THESIS ADVISOR : SOMSAK PANTUWATANA, Ph.D., CHALOBON YOOSOOK, Ph.D. 77 p. ISBN 974-588-902-4

Inactivated Japanese encephalitis (JE) vaccines of mouse brain origin are widely used today in Thailand. These vaccines contain high rates of foreign proteins that are associated with special risk categories, such as side reactions of postvaccine encephalitis. Vaccines of cell culture origin are developed to avoid the hazards associated with that protein. However, the yields of JE virus in monolayer culture are quite low.

Two culture systems, suspension cell culture and microcarrier culture, were developed for cultivation of BHK-21 cells. The microcarriers were prepared by derivatization of Sephadex G50 with DEAE. It was found that these microcarriers supported the attachment and growth of BHK-21 cells on their surfaces. The BHK-21 cells attached on microcarriers multiplied slower than the BHK-21C13L18 cells grown in suspension culture system. However, the microcarrier culture system gave higher final cell densities than that of suspension culture system. The multiplication ratio of cells in microcarrier culture system was 7.5 while it was 6.6 in suspension culture system.

The JE virus was grown in these two systems with the M.O.I. of 0.1, and the virus yields were determined by microplate plaque assay. The virus productions in suspension culture system were higher than those produced in the microcarrier culture system. The virus titers were increased to $5.05X10^7$ PFU/ml at 48 hours post-infection in suspension culture system, and $1.09X10^7$ PFU/ml at 24 hours post-infection in microcarrier culture system. The virus titers in both culture systems were stably maintained until 96 hours post-infection. It was found that the virus titers in conventional monolayer culture gradually declined after they reached the highest titer of $1.06X10^7$ PFU/ml at 48 hours post-infection. Thus, it is possible to develop these two cell culture techniques for the multiplication of JEV to manufacture vaccines.