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Short Communication

**DEVELOPMENT OF A PRACTICABLE METHOD FOR ISOLATION
AND IDENTIFICATION OF DENGUE VIRUSES IN DEVELOPPING
COUNTRIES**

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SUMMARY: We sought a practicable method for isolation and identification of dengue viruses in South-East Asia. We compared two mosquito cell lines, C6/36 and TRA-284-SFG, for virus isolation and two identification methods, immunofluorescent staining of infected cells with serotype-specific mouse monoclonal antibodies and a sandwich-type enzyme-linked immunosorbent assay with conventional mouse hyperimmune ascitic fluids. We found that the combination of TRA-284-SFG cells and ELISA is a useful and feasible method in developing countries.

There are many techniques available for isolation and identification of dengue viruses: for virus isolation, inoculation of the specimen into suckling mouse brains (1), into the thorax of *Aedes* (2) or *Toxorhynchites* (3) mosquitoes, or into vertebrate (4) or mosquito (5) cell lines; for serotype identification, complement fixation (6), immune adherence hemagglutination (7), indirect fluorescent antibody (IFA) staining with monoclonal antibodies (8), and enzyme-linked immunosorbent assay (ELISA) with conventional mouse hyperimmune ascitic fluids

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(9) or monoclonal antibodies (10).

In tropical countries where dengue viruses are prevalent, however, the adoption of these techniques may be hampered by the circumstances of virus laboratories there, e.g. the lack of equipment, and skilled personnel and the high cost. Therefore, we tried to develop a suitable system for the dengue virus study feasible in the Philippines.

In this study, we compared two mosquito cell lines, C6/36 (11) and TRA-284-SFG (12), for virus isolation, and two identification methods, IFA (8) and ELISA (9), to find a most suitable combination. TRA-284-SFG cell line was a subline of TRA-284-SF derived from the first instar larvae of a nonbiting mosquitoes, *Toxorhynchites amboinensis*, and maintained in a serum-free medium consisting of equal volumes of Leibovitz-15 and tryptose phosphate broth. Cells were cultured in glass culture tubes. One-fifth-milliliter quantities of acute phase human sera diluted 1:10 with Hank's balanced salt solution supplemented with 2% fetal bovine serum, 0.5% lactalbumin hydrolysate and 100 µg per ml of DEAE-dextran in 0.01 M Tris-HCl, pH 7.4, were inoculated onto cell layers. After the adsorption of virus at 28 C for 60 min, 2.0 ml of the maintenance medium was added to the tube cultures without removing the inocula. After 7-day incubation at 28 C, infected cells and culture fluids were harvested separately for IFA staining and ELISA. At the same time, 0.2-ml quantities of infected culture fluids were inoculated to other cultures for the second passage. Harvested cells were washed twice with phosphate-buffered saline and allowed to attach by air drying on the slide glass and then to react with serotype-specific mouse monoclonal antibodies and fluorescein-conjugated goat anti-mouse IgG serum. Mouse monoclonal antibodies, coded as 15F3, 3H5, 5D4 and 1H10, for dengue type 1 (Hawaii strain), type 2 (New Guinea C), type 3 (H87) and type 4 (H241), respectively (8), were provided by Dr. N. Karabatsos, Centers for Disease Control, U.S.A. We carried out also sandwich ELISA (9) for detection of the viral antigens present in infected culture fluids.

From April, 1986 till March, 1987, we processed a total of 275 human sera in Virology Section, Research Institute for Tropical Medicine, Department of Health, Republic of the Philippines. We finally isolated 39 strains of dengue viruses including seven strains of dengue type 1, 16 strains of type 2 and 16 strains of type 3 viruses from the Metro Manila area, Davao in Mindanao and Bohol. The number of viral antigen-positive cultures given in Table I does not coincide with the above-mentioned number of dengue virus isolates as multiple specimens were taken from some patients. The rate of viral antigen-positive cultures was higher by IFA staining than by ELISA both in C6/36 and TRA-284-SFG cells. However, the IFA

Table I. Comparison of two cell lines for dengue virus isolation

	TRA-284-SFG cells		C6/36 cells	
	by ELISA	by IFA	by ELISA	by IFA
Number of specimens tested*	269	242	194	128
Number of virus-positive cultures	47	53	18	27

*Total number of the specimens received was 275.

method was less practicable than the ELISA technique, because the former was more time-consuming and often affected by cell damages; we were able to handle 269 of the 275 specimens (97.8%) by the combination of TRA-284-SFG cells and ELISA but only 128 of the 275 specimens (46.5%) by the combination of C6/36 cells and IFA staining. We often failed to carry out IFA staining with C6/36 cell cultures owing to the cytotoxic activity of human sera, which caused detachment of the cell layers from the glass wall and death of cells within 2 to 3 days after inoculation of the virus. The cytotoxic reaction was stronger in C6/36 cells than in TRA-284-SFG cells, even if we removed the inoculum after adsorption of the virus. Kuno et al. (13) reported similar results on the cytotoxicity of human sera against cultured mosquito cells such as C6/36. The results of serotype determination by ELISA completely agreed with those by IFA staining.

From the above data and our experiences, we conclude that the combination use of TRA-284-SFG cells and ELISA is a practicable method for dengue virus isolation. First, TRA-284-SFG cell line does not require addition of fetal bovine serum to the growth or maintenance medium. The use of fetal bovine serum required for C6/36 cell is a heavy financial burden in developping countries. Second, TRA-284-SFG cells are more resistant to the cytotoxicity of human sera than are C6/36 cells. Third, the time required for ELISA is only half or less of that for IFA staining for a given number of samples. In an outbreak of dengue, it is necessary to isolate and identify the agent as quickly as possible. Fourth, ELISA is

done with the use of easily available conventional hyperimmune mouse ascitic fluids and does not require serotype-specific mouse monoclonal antibodies.

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