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A RAPID METHOD FOR THE ISOLATION AND IDENTIFICATION OF DENGUE VIRUSES EMPLOYING A SINGLE SYSTEM*

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Methods currently in use for the isolation and type-identification of dengue viruses are time-consuming and require the employment of more than one system. We are now testing Varma's *Aedes pseudoscutellaris* cell line' grown in Lab-Tek slides' as a single system for rapid isolation and typing. Our results to date show that this is a sensitive and rapid method for this purpose.

We adapted a culture of *Aedes pseudoscutellaris* cells, received from Dr. Goro Kuno of the San Juan Laboratories, Puerto Rico, for adherence to glass surfaces. The adapted cells are pipetted into four-chamber Lab-Tek slides and reach 70–80% confluency usually within 24 hours. An appropriate dilution of the specimen, from which virus is to be recovered, is then added to each of four chambers. Human serum, which we have found to be toxic to mosquito cells in low dilutions, is used at a dilution of 1:20, which eliminates the toxic reaction. After incubation at 27°C., the media are poured off and the plastic chambers removed from the glass slides, which are then fixed for 20 minutes in cold acetone. The fixed slides may be stored frozen or used immediately in the indirect fluorescent antibody technique. Mouse immune sera for each of the four dengue virus types, previously titrated for fluorescent endpoints on dengue-infected control slides, are added to the fixed slides, one chamber for each type-specific serum. After incubation and washing, anti-mouse goat serum fluorescein conjugate is applied with a final incubation and careful washing before examination under the fluorescent microscope. Each test includes positive and negative controls.

Prototype strains of the four types of dengue virus were all readily identified in blind tests. To date, 58 strains of dengue have been isolated and identified by this method. Virus identifications were confirmed by plaque reduction neutralization tests on Vero cells. Twenty-nine isolates were made from human sera; two from monkey sera; and three from mosquito pools. These isolations include all four dengue types from human sera and three of the types from mosquito pools.

This method has the advantage of requiring only one system from isolation to final identification. Mouse or mosquito colonies need not be maintained. Observation for cytopathic effects and syncitial formation in the cultures, which may be seen, is not necessary, since the final examination for fluorescence is highly sensitive and clearly observed.

Detailed Methods

Approximately 2.5 to 2.7 x 10^5 glass grown *Aedes pseudoscutellaris* (Aps) cells suspended in Mosquito-VP12 (Aps) medium + 20% foetal calf serum (FCS) are inoculated into each chamber of a four-chamber Lab-Tek slide. Three slides are used for each isolation attempt. The slides are incubated overnight at 28°C. The following day the medium is poured off each slide and infected with a suspect virus specimen; 0.15 ml of appropriately diluted virus suspension is added to each chamber. The slides are incubated at 28°C. for 1½ hrs, then brought up to a 1 ml volume with Aps medium + 2.5% FCS.

Pig Kidney (PS) cells and LLC-MK_2 cells at approximately the same inoculating suspension have also been used in this system.

Human sera must be diluted to approximately 1:20 to prevent cytotoxic effects on mosquito cells. Suckling mouse brain suspensions are usually diluted to 2 x 10^{-2} or higher depending on the particular stock and/or pre-

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vious titration. Mosquito pool suspensions, arbitrarily diluted, are centrifuged at 15,000 rpm for 15 minutes prior to inoculation.

Fluorescent Antibody Staining: One slide is taken at day 4 or 5 and another taken at day 10 following infection. The media are poured off and the plastic upper chamber carefully removed, leaving the rubber gasket attached to the slide. The slides are fixed for 10 minutes in ice cold acetone, then air dried. The slides are then used immediately or stored in the freezer for later staining. The indirect method of fluorescent antibody staining is used for identification. Appropriately diluted hyperimmune mouse serum (0.15 ml) is added to each chamber of the infected slide. Each slide receives all four types of dengue antiserum, one type to each chamber. The slides are incubated for 45 minutes at 37°C. in a humidified atmosphere. After incubation the excess sera are poured off the slides, which are then washed twice in phosphate buffered saline (pH 7.5). The slides are drained and 0.15 ml of fluorescein conjugated goat anti-mouse globulin at the appropriate dilution is placed in each chamber. Again the slides are incubated for 45 minutes at 37°C. and washed twice. The rubber gasket is removed carefully and each slide mounted with buffered glycerol (pH 9.0) and a cover glass.

A virus isolate was deemed a particular type of dengue virus if it gave 3+ or better fluorescence in one particular chamber and 1+ or less in the other chambers. Fluorescence must be observed in all four chambers in order to ascertain that infection was successful in each.

Harvesting: If the virus identification is inconclusive or if only some of the chambers show infection, the third slides in the series is harvested. Small glass beads are added to each chamber and rolled gently to break up the cell sheet. The suspension is removed and centrifuged for clarification and either passed immediately or stored in ampoules at −70°C. for subsequent passage.

REFERENCE

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