Increased Detection Rate for Varicella-Zoster Virus with Combination of Two Techniques

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Standard cell culture and centrifugation culture were compared for the isolation of varicella-zoster virus from 337 clinical specimens. Of a total of 85 (25%) positive specimens, 67 (78.8%) were positive by centrifugation culture alone and 18 (21.2%) were positive by both methods. When epithelial cells from the specimen were included in the vial inoculum, these cells stained positive and increased the varicella-zoster virus detection rate by 30%.

Varicella-zoster virus (VZV), the causative agent of chickenpox and shingles, is difficult to propagate in standard cell cultures. The virus is known to be highly cell associated, and intimate contact between infected and noninfected cells is necessary for growth (1, 2). This contact is most probably maximized in the centrifugation-enhancement culture method which has been found by several investigators (C. A. Gleave, C. I. Bustamante, C. F. Lee, R. A. Bowden, and J. D. Meyers, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C252, p. 365), as well as ourselves, to be more sensitive for VZV detection than standard culture. A third technique for identifying VZV in clinical specimens is direct immunofluorescent-antibody staining, a method in which the specimen is centrifuged and cells are collected, spotted on a slide, and stained with fluorescein-conjugated anti-VZV serum. This method also has been shown to be more sensitive than standard culture (Gleave et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C252, p. 365). In this paper, we examine the possibility of combining centrifugation culture and direct immunofluorescent-antibody staining.

This is made possible by including epithelial cells from the specimen, if present, in the shell vial inoculum and proceeding with the centrifugation. The epithelial cells from the specimen adhere to the MRC-5 monolayer on the cover slip and, if positive for VZV, are stained by the anti-VZV monoclonal antibodies at the same time as the monolayer itself.

MRC-5 (human embryonic fibroblast) cultures grown on cover slips in shell vials or in tubes were obtained from Whittaker Bioproducts, Walkersville, Md. Eagle minimum essential medium containing 2% fetal bovine serum, 1% glutamine, 50 mg of gentamicin per liter, 50 mg of vancomycin per liter, and 2.5 mg of amphotericin B (Fungizone) per liter was used throughout. Medium, serum, and gentamicin were from Whittaker Bioproducts; vancomycin was from Sigma Chemical Co., St. Louis, Mo.; and glutamine and amphotericin B were from Flow Laboratories, Inc., McLean, Va.

Fluorescein-conjugated monoclonal antibodies to VZV were obtained from Ortho Diagnostics, Raritan, N.J.

Clinical specimens were obtained from cutaneous lesions and submitted to our facility for isolation of VZV. The positive control virus was isolated in our laboratory from a clinical specimen.

Clinical specimens were received in viral transport media (SmithKline Bio-science). They were vortexed with glass beads for 30 s and allowed to settle for approximately 1 h. Shell vials were inoculated (two per specimen) with 0.2 ml of Eagle minimum essential medium and a 0.2-ml specimen inoculum containing epithelial cells from the bottom of the vial. A positive and a negative control (Eagle minimum essential medium) were included in each test run. The vials were centrifuged (1,000 x g; Beckman J6-B) for 1 h at 36°C and were then incubated at 36°C for 48 h. Cover slips were stained in the vial by the direct method, mounted on slides, and read with a UV microscope.

For the standard cell culture method, specimens were handled as in the centrifugation-enhancement method described above. MRC-5 tubes (two per specimen) were inoculated with 0.2 ml of specimen inoculum per tube. The tubes were held at 36°C for 14 days and read daily for VZV-specific cytopathic effect. Positive control virus was inoculated with each group of specimens.

In this study, 337 specimens were tested for the presence of VZV. Of these, 85 specimens or 25% of the total were positive. All of the 85 positive specimens were detected in the shell vial assay, but only 18 specimens or 21.2% were detected in standard culture tubes. The 85 positive specimens in the vials could be further separated into those in which the monolayer cells were positive (31%), those in which only the specimen-derived epithelial cells stained positive (31%), and those which exhibited both monolayer staining and specimen-derived epithelial cell staining (38%). In Fig. 1 are shown the different types of positive VZV staining encountered when specimen-derived epithelial cells were included in the shell vial inoculum.

VZV is very difficult to grow in cell culture. In 5 to 7 days after inoculation, it produces small focal patches of cytopathic effect which often stop spreading and disintegrate after several days. It is for this reason that virology laboratories have sought quicker, more sensitive methods for detecting VZV. The slide direct immunofluorescent-antibody staining method and the centrifugation-enhancement method both satisfy these requirements. In this study, we
FIG. 1. Staining patterns seen on VZV cover slips. (A) Single infected cell; (B) infected plaque in the monolayer; (C) specimen-derived epithelial cells on top of the monolayer; (D) combination of an infected monolayer plaque and supramonolayer specimen-derived epithelial cells. Magnification, ×100.

We attempted to combine the advantages of both these methods into one test and found that by doing so, we increased our VZV detection rate by 30%.

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LITERATURE CITED