Evaluation of an Enzyme-Linked Immunosorbent Assay for the Detection of Herpes Simplex Virus Antigen

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An enzyme-linked immunosorbent assay (ELISA) kit for herpes simplex virus developed by Ortho Diagnostic Systems, Inc., was evaluated. In phase I experiments, 263 clinical specimens from genital lesions were extracted into serum-free medium and then tested by ELISA for herpes simplex virus antigen. The results were compared with those obtained by conventional viral culture. Of 83 specimens, 65 were positive by ELISA (sensitivity, 78.3%). In phase II experiments, 249 clinical specimens were tested for herpes simplex virus antigen in direct specimen and in cell cultures (MRC-5 and rabbit kidney) incubated for 2, 4, and 7 days. Of 63 specimens, 40 were positive by ELISA in the direct specimen (sensitivity, 63.5%), and by 7 days incubation, 100% of the cultures positive by viral cell culture were also positive by ELISA. The ELISA was reproducible, and when both the direct detection and amplification culture were used, the sensitivity of ELISA paralleled the diagnosis of herpes simplex virus infections by viral cytopathic effect.

Enzyme-linked immunosorbent assay (ELISA) has become a routine procedure in clinical laboratories for the detection of many antigens and antibodies. However, to date ELISA has only been applied experimentally to detect herpes simplex virus (HSV) antigen (1, 2). These assay systems were very specific (specificities ranging from 96 to 100%) but lacked sensitivity (sensitivities ranging from 46 to 76%) in detecting HSV antigen directly from clinical specimens when compared with viral isolation.

In contrast, Vestergaard and Jensen (3) have developed an ELISA system for HSV antigen detection that is reportedly more sensitive than viral culture methods. They found 8 of 56 specimens to be positive by ELISA but negative in cell culture. Since no confirmation test was used, these eight specimens could have given false-positive results.

These reports only discuss the usefulness of ELISA HSV antigen detection for direct clinical specimen testing. No clinical studies have been reported concerning antigen testing after virus cultivation in a cell culture system. Supplementation of direct antigen detection with a viral amplification system should increase the overall sensitivity of HSV antigen detection. Also, a reliable two-step HSV antigen detection method based on ELISA could be used by a microbiology laboratory unfamiliar with virology techniques.

This report is an evaluation of a commercial kit developed by Ortho Diagnostic Systems, Inc., for the detection of HSV antigen from clinical specimens and two conventional cell culture systems. In this study, the ELISA was evaluated for specificity, sensitivity, and speed of HSV antigen detection as compared with conventional viral culture methods.

MATERIALS AND METHODS

Clinical specimens. Clinical specimens were obtained from patients with symptoms suggestive of a genital HSV infection. One specimen was collected from each patient by using a Culturette swab. Specimens were inoculated either immediately or after transport at 4°C. Approximately 75% of the specimens were 12 to 24 h in transport. For virus cultivation and ELISA testing the swab was rotated vigorously and thoroughly drained in 2.2 ml of serum-free (SF) medium (Eagle serum-free maintenance medium with antibiotics) for phase I experiments, or in 3.0 ml of SF medium for phase II experiments. A portion (0.5 ml) of the SF medium extract was pipetted into the swab cylinder to collect any residual virus and then removed and returned to the SF medium vial. After virus cultivation, the remaining SF medium extract was stored at −70°C until ELISA testing. All specimen processing was done in a biological safety cabinet.

Viral isolation. (i) Phase I. MRC-5 cells (Flow Laboratories) were used for virus isolation. The SF medium (0.25 ml) was added to one tube culture per patient specimen. Cultures were incubated at 35°C for 7 days, and the tubes were observed daily for cytopathic effects (CPE). The direct specimens were tested by ELISA in a blind fashion without knowledge of culture results.

(ii) Phase II. MRC-5 and primary rabbit kidney (RK; Flow Laboratories) cells were used for viral isolation. The SF medium extract (0.25 ml) was added to three MRC-5 tube cultures and three RK tube cultures per patient specimen. One culture tube of each cell type was incubated at 35°C for 2, 4, and 7 days. The cultures were observed daily for CPE. If CPE was observed, the culture tube was frozen at −70°C until ELISA testing. After designated incubation periods, the culture tubes were frozen at −70°C until ELISA testing. All culture tubes were assayed within a 6-week time period. Each patient specimen was eventually tested by the following protocol: direct specimen; MRC-5 (days 2, 4, and 7); RK (days 2, 4, and 7); and pooled specimen (MRC-5 plus RK cell culture) for days 2, 4, and 7 postinfection.

ELISA. The assay kit contains Removawell strips (Ortho) coated with herpes simplex antibody and blocked with protein stabilizer. Clinical specimens in SF medium (0.2 ml) were dispensed into individual wells and incubated for 2 h at room temperature. All direct specimens in SF medium for direct assay were tested in duplicate, and all culture tubes were tested singly. The wells were washed three times with washing solution (Ortho), and then 0.2 ml of horseradish peroxidase-conjugated herpes simplex antibody (Ortho) was added to each well. After incubation at room temperature for 2 h, all wells were washed five times with washing solution.

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and 0.2 ml of o-phenylenediamine (Ortho) Diagnostic was added. The reaction mixture was stopped with 0.05 ml of 2 N HCl (analytical grade) after 30 min of incubation. The optical density was read on a Dynatech micro-ELISA minireader (MR 590) at 490 nm.

In each assay, a substrate blank, SF medium control, and low-titer virus and high-titer virus controls (Ortho) were assayed. In this study, an ELISA result was determined to be positive if an optical density reading equal to or greater than that of the low-titer virus control was obtained.

RESULTS

Phase I experiments. Detection of HSV in clinical specimens by cell culture isolation and ELISA. Viral isolation and ELISA were performed with 263 clinical specimens from patients with symptoms suggestive of an HSV infection. Eighty-three specimens (31.6%) were positive for HSV in cell cultures. The sensitivity of the ELISA assay was 78.3%; 18 of the 83 HSV positive isolates were not detected by this method (Table 1). Since the commercial kit will not contain a low-titer virus control, a result will be considered positive if a net optical density reading (net = patient reading – average transport control reading) is equal to or greater than 0.15 times the net high-positive virus control average.) Recalculation of results using this criterion for identifying positive specimens gave a sensitivity for direct antigen detection of 72.3%. The mean time for CPE to develop was 5.0 days for the specimens positive by culture and negative by ELISA as compared to 2.5 days for those positive in both assays. There was no instance in which the ELISA assay was positive and the culture result did not yield HSV (specificity, 100%).

Phase II experiments. Detection of HSV in clinical specimens by an amplification culture system and ELISA. Virus amplification culture and ELISA were performed with 249 clinical specimens from patients with symptoms suggestive of genital HSV infection. Sixty-four specimens (25.7%) were positive by virus isolation in cell cultures. ELISA of direct specimens detected viral antigen in only 63.5% of HSV culture-positive specimens (Table 1). The recalculated sensitivity based on the transport control value was 60.6%. The mean time for CPE to develop was 2.7 days for the 40 HSV culture- and ELISA assay-positive specimens and 4.8 days for the 23 HSV ELISA-negative specimens. The ELISA was positive only when cell culture results yielded HSV (specificity, 100%).

Amplification culture tubes incubated for 2, 4, and 7 days were assayed for the presence of HSV antigen. There was very little difference in the sensitivities of the MRC-5 and RK cell cultures for the development of characteristic CPE due to HSV and for the detection of HSV by the ELISA (Table 2). The number of positive specimens by the ELISA correlated directly with the time period of incubation, with the 7-day tube having the greatest number of positive specimens. Also, the maximum number of positive specimens was detected by ELISA with the pooled specimens (MRC-5 plus RK cell culture tubes) as compared with the individual culture tubes. Complete agreement between viral isolation and ELISA was obtained with the 7-day RK culture tube and the 7-day pooled specimen. Also, ELISA with direct and amplification culture testing was significantly more rapid than CPE for the detection of HSV antigen (Fig. 1). Statistical significance was demonstrated by the sign test and paired t test (P < 0.001).

DISCUSSION

The comparison of HSV antigen detection by ELISA and viral culture indicated that ELISA testing of the direct specimen SF extract coupled with the amplification system was a reliable alternative to conventional viral isolation in cell cultures. The ELISA technique requires minimal reagent preparation and results are available within 5 h. Although this procedure is technically demanding, rapid and reliable diagnostic information is provided. The technical skills required are those inherent to all ELISA systems (accurate pipetting and uniform washing procedures) and are particularly necessary for the reproducibility of this test. Following strict laboratory procedures, we determined the interassay variability for a high- and low-titered sample in
duplicate in 11 different assays. The variance was 0.04 and 0.01 for the high and low sample, respectively. Only minimal experience in viral techniques involving cell cultures is needed to implement the virus amplification system, making this ELISA method applicable to bacteriology laboratories who want to provide diagnostic tests for detecting HSV.

The ELISA test kit was not as sensitive as conventional viral isolation for the direct detection of antigen in clinical specimens. The direct antigen detection rates in this evaluation, 78.3 and 63.5%, are in the range of earlier reports of Grillner and Landquist (1) and Miranda et al. (2). Noteworthy was the decrease of ca. 15% in antigen detection in phase II as compared to phase I of this study. We feel that this is directly attributable to the amount of SF medium used for specimen extraction; phase I used 2.2 ml and phase II used 3.0 ml of SF medium. Therefore, the initial volume of SF medium used for specimen extraction is a critical factor to consider in using this ELISA. An additional decrease in the volume of specimen extraction medium should theoretically afford a greater sensitivity in the direct antigen detection assay.

The majority of our patient specimens were transported for several hours at 4°C. This delay could have resulted in a lowered titer of active virus at the time of inoculation of cell cultures and hence an extended time for the detection of CPE. The time range for the development of CPE in this study was 1 to 7 days with a mean time of 3.5 days. ELISA was more rapid in the amplification systems than was the detection of CPE in 58 of the 63 specimens containing HSV (Fig. 1).

Thus, ELISA of MRC-5 and RK cell culture tubes after 4 days of incubation missed only 2 and 1 positive specimens, respectively (Table 2). Interestingly, the single ELISA-negative MRC-5 cell culture tube (7 days) was selective for growth in RK cells. Nevertheless, the added expense and workload of processing two cell culture systems for a net gain in sensitivity of less than 2% is a question that each laboratory must evaluate. Alternatively, the pooled cell culture lysates of MRC-5 plus RK cell cultures provides maximum sensitivity and requires only one ELISA assay.

This is the first ELISA detection system for HSV to be developed commercially. Since HSV is the most common isolate in almost every diagnostic setting, this ELISA kit could be very helpful to clinical laboratories. Perhaps as important as rapid testing time is the implementation of the direct detection and amplification culture method as an alternative to standard viral culture. This system is reliable, and although direct detection could not be used alone, when used with amplification culture, the sensitivity of ELISA paralleled results of viral CPE assays.

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LITERATURE CITED

