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Multisite Evaluation of a Monoclonal Antibody Reagent (Syva) for Rapid Diagnosis of Cytomegalovirus in the Shell Vial Assay

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A pre-cytopathic effect (CPE) monoclonal antibody reagent (Syva Co., Palo Alto, Calif.) was evaluated in four laboratories for the rapid detection of cytomegalovirus (CMV) in shell vial cell cultures at 16 to 24 h and 40 to 48 h postinoculation. Results were compared with those obtained by inoculation of the specimen into conventional tube cell cultures that were examined for the presence of typical CMV CPE and subsequently tested by reaction with the monoclonal antibody reagent in an indirect immunofluorescence test. Of 937 specimens, CMV was positive in 184 (20%). CMV was detected twice as frequently in shell vials only ($n = 29$) as in conventional tube cell cultures ($n = 14$). Pre-CPE shell vial assay was 91% sensitive (range, 84 to 98%) and 96% specific (range, 93 to 98%) compared with the detection of CPE in conventional tube cell cultures. Overall, 137 of 166 (83%) and 143 of 166 (86%) of the CMV strains were detected at 16 to 24 h and 40 to 48 h postinoculation, respectively. The Syva reagent produced sensitive and specific results for the rapid detection of CMV infection in shell vial cell cultures and reliably confirmed the presence of the virus as detected by CPE in conventional tube cell cultures.

Cytomegalovirus (CMV) causes severe infections in immunocompromised (immunosuppressed) patients, such as transplant recipients, congenitally infected infants, and patients with acquired immunodeficiency syndrome (AIDS) (3, 15). Because of the expanding importance of CMV in clinical medicine, rapid diagnostic methods are needed for the routine detection of CMV in the clinical laboratory. Early detection is especially critical now that an antiviral agent, ganciclovir [DHPG; 9-(1,3-dihydroxy-2-propoxymethyl)guanine], is available for the treatment of CMV infection (9). Conventional cell culture isolation techniques generally have required 7 to 28 days postinoculation for recognition of characteristic cytopathic effect (CPE) (7, 16). In the past few years, several laboratories have described the use of the shell vial assay for the rapid detection of CMV infections (pre-CPE) (1, 2, 4, 5, 10, 13). The method is based on the enhancement of CMV infection in cell monolayers on cover slips in shell vials after low-speed centrifugation. After incubation for 16 to 48 h, CMV infection is detected by an indirect immunofluorescence test using a monoclonal antibody directed to an immediate-early (72,000-dalton) antigen of the virus. Syva Co. (Palo Alto, Calif.) has developed a reagent containing monoclonal antibodies to both the immediate-early antigen of CMV and an early antigen (50,000 daltons) of the virus. The purpose of this study was to evaluate this product in four diagnostic virology laboratories for the detection of CMV in the shell vial assay and as a reagent for confirming the presence of the virus in conventional tube cell cultures as detected by CPE.

MATERIALS AND METHODS

Specimens and patient population. Specimens were obtained from 937 patients (663 [71%] male, 250 [27%] female, and 24 [2%] gender unknown). The study population comprised organ transplant (240 [26%]), acquired immunodeficiency syndrome (170 [18%]), newborn (117 [12%]), pediatric (27 [3%]), and unknown (383 [41%]) patients.

Trial sites. The general experimental design required that all test sites control the following variables in the shell vial assay: cell line (MRC-5), inoculation volume (0.3 ml/vol), passage number, centrifugation force (700 × *g* for 40 min at 20 to 30°C), time of staining of infected cell culture monolayers with the pre-CPE reagent (16 to 24 and 40 to 48 h postinoculation), and staining procedure. Trial sites 1 and 2 prepared shell vial cell cultures in their laboratories, whereas sites 3 and 4 purchased these cultures from commercial vendors. Conventional tube cell cultures were inoculated with 0.25 ml per tube and were examined daily for at least 28 days for evidence of CMV CPE. Human foreskin fibroblasts and WI-38 cells (site 3) were used by three of the four trial sites (site 1 used MRC-5 cells).

Specimen inoculation. Positive (e.g., AD169, Towne, or Davis strains) and negative controls were inoculated at the same time for each batch of shell vials and tube cultures processed. Shell vials and conventional tube cell cultures were inoculated on the same day. After removal of the maintenance medium, 0.3 ml of specimen was inoculated into each of two shell vials and 0.25 ml was inoculated into one or two routine culture tubes. Each laboratory followed its own procedure for detection of CPE due to CMV in routine tube cell cultures.

After addition of the inoculum to the shell vials, the vials were centrifuged at 700 × *g* for 40 min at 20 to 30°C. After centrifugation, 2.0 ml of maintenance medium (prewarmed to 37°C) was added to each vial, and the vials were placed in an incubator at 37°C. One vial was processed for staining after 16 to 24 h, and the other was processed after 40 to 48 h.

Staining procedure for shell vial cultures. At 16 to 24 h postinoculation, the medium was removed from the vial, and the monolayers were rinsed twice with 1.0 ml of phosphate-buffered saline (PBS). After removal of the PBS, the monolayers were fixed with 2.0 ml of acetone for 10 min at room

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TABLE 1. Detection of CMV in shell vial cell cultures according to specimen type

Specimen type	No. of specimens with CMV detected at trial site:				% of total specimens	No. of positive cell culture results in:		
	1	2	3	4		Shell vials and conventional tube	Shell vials	Conventional tube
Urine	155	22	141	59	40	91	9	7
Throat swab	14	19	3	0	4	4	1	0
Bronchoalveolar lavage	9	7	4	8	3	6	3	1
Bronchial aspirate/sputum	3	1	40	20	7	15	5	1
Tissue (liver, lung, stomach, rectum, esophagus, duodenum)	8	63	30	22	13	12	7	1
Rectal swab/stool	11	3	7	3	3	0	1	0
Blood (buffy coat)	0	1	142	82	24	10	5	4
Cerebrospinal fluid	0	0	15	9	3	0	0	0
Fluid (pericardial, pleural, tracheal aspirate)	0	0	10	4	1	1	0	2
Bone marrow	0	0	6	1	1	0	0	0
Other	0	1	6	8	1	2	0	0

temperature. After aspiration of the acetone, the monolayers were rinsed with 1.0 ml of PBS to ensure even distribution of the staining reagents. Anti-CMV monoclonal antibody reagent (150 μl) was added to each cover slip. The vials were recapped and placed at 37°C for 30 min. The reagent was aspirated, and the monolayers were rinsed with two 1.0-ml portions of PBS. After aspiration of the PBS, 150 μl of fluorescein-conjugated goat anti-mouse immunoglobulin G (IgG) reagent was added to each cover slip monolayer, and vials were recapped and placed at 37°C for 30 min. This reagent was removed, and each monolayer was washed twice with 1.0 ml of distilled or deionized water. The cover slip was gently removed from the vial by using a needle and forceps and placed cell-side down on a drop of mounting fluid (Syva).

Evaluation of shell vial cover slips and routine culture smears. Cover slips and smears were considered positive for CMV if at least one intact cell with typical intranuclear immunofluorescence staining was present. Cover slips and smears were considered negative for CMV if adequate cellular material was present (at least 70% of the cover slip monolayer or at least 100 intact cells on the routine culture smear) and no cells showed typical CMV fluorescence staining.

Staining procedure for routine culture confirmation. Routine cultures were processed only when at least 10 to 50 foci of CPE typical for CMV were present. The medium was removed, and the cell monolayer was washed with 2 ml of PBS. This wash reduced the fetal bovine serum present on the monolayer. After the PBS was aspirated, 0.5 ml of PBS was added to each tube and the monolayer was scraped into the buffer with a transfer pipette or plastic scraper. The cells were dispersed, and the suspension was centrifuged for 15 min at 300 × *g* at room temperature. The cell pellet was suspended in 100 to 200 μl, and 25 μl was placed in two smears in 8-mm-diameter slide wells. One slide well was stained with either Bartel or Serono CMV culture confirmation reagent. The other slide well was to be stained with the pre-CPE monoclonal antibody reagent (Syva Co). The smear was completely air dried and fixed by immersion in acetone for 10 min. After the acetone was completely evaporated, the slide was stained by adding 30 μl of Reagent 1 (anti-CMV monoclonal antibodies) and incubated for 30 min at 37°C in a moist chamber. Excess reagent was removed, and the slide well was rinsed with a gentle stream of PBS from a wash bottle for 10 s. Excess PBS was removed, and 30 μl of Reagent 2 (fluorescein-conjugated goat anti-mouse IgG) was added to the slide well prior to incubation in a moist chamber for 30 min at 37°C. After excess reagent was removed, the slide was rinsed with a gentle stream of distilled water from a wash bottle for 10 s. The slides were air dried, a drop of mounting fluid was added to each well, and a cover slip was applied.

RESULTS

Urine (377 [40%]), respiratory sites (bronchial and throat) (128 [14%]), and blood (225 [24%]) represented 78% of the specimens and 86% (urine, 57%; respiratory, 19%; blood, 10%) of the CMV strains detected (Table 1). Overall, CMV was detected in 184 of 937 (20%) specimens. The monoclonal antibody reagent of Syva Co. produced a bright homogeneous intranuclear fluorescence that reacted specifically with CMV and not with other herpesviruses (herpes simplex virus and varicella-zoster virus).

Of 184 CMV strains, 141 (77%) from 937 specimens were positive in both conventional tube and shell vial cell cultures. Thirty-six specimens were evaluable in only one culture system due to contamination or toxicity problems; these were included in the analysis as negative samples. Seven of these specimens were CMV positive (five in shell vial cultures and two in conventional tube cell cultures). Twice as many CMV-positive results were obtained exclusively in the pre-CPE shell vial assay (29 specimens) compared with recognition of CPE in conventional tube cell cultures (14 specimens). For purposes of analysis, the conventional tube cell culture method was assumed to be the standard. The pre-CPE shell vial test was 91% sensitive (range, 84 to 98%) and 96% specific (range, 93 to 98%) compared with the detection of CPE in conventional tube cell cultures.

Trial sites 1 and 2 seeded their own shell vials with MRC-5 cells and inoculated monolayers 3 to 8 days after preparation. Trial sites 3 and 4 purchased preseeded MRC-5 shell vials and inoculated them 8 to 15 days after preparation by the commercial vendor. At all sites, each patient specimen was inoculated into two shell vials; one shell vial was incubated for 16 to 24 h and the other was incubated for 40 to 48 h. Of 71 CMV strains detected at trial sites 1 and 2, 69 (97%) were detected at 16 to 24 h (Table 2). Only 68 of 95 (72%) CMV strains were detected at 16 to 24 h at the trial sites that used commercially prepared shell vial cell cultures. The data suggested that the sensitivity of this system for next-day testing depended on the use of young monolayers at the time of specimen inoculation.

TABLE 2. Detection of CMV in laboratory-prepared and in preseeded commercial shell vial cell cultures

Trial sites	No. of specimens with the results[a]:			No. (%) positive with pre-CPE staining at:	
	16–24+/ 40–48+	16–24+/ 40–48−	16–24−/ 40−48+	16–24 h	40–48 h
1 and 2[b]	53	16	2	69 (97)	55 (77)
3 and 4[c]	61	7	27	68 (72)	88 (93)

[a] CMV-positive (+) and -negative (−) results obtained after staining with the pre-CPE reagent after 16 to 24 and 40 to 48 h.
[b] Shell vials prepared in site laboratory.
[c] Shell vials prepared by commercial vendors.

To evaluate the effectiveness of the reagent (pre-CPE) for use as a confirmatory test to identify CMV detected by CPE, conventional tube cell cultures containing 10 to 50 plaques or foci were harvested. The cell suspension was centrifuged, and the pellet was used to prepare smears in 8-mm slide wells; the slides were then stained with the Syva CMV reagent. Fluorescent foci were observed in 140 of 147 (sensitivity, 95%) smears from intact cells of tube cell cultures with CPE typical for CMV infection (Table 3).

DISCUSSION

The pre-CPE monoclonal antibody reagent developed for the rapid immunological detection of CMV provided for an early and accurate laboratory diagnosis of these infections. Of 937 evaluable specimens, from which 184 CMV strains were detected, 29 that did not yield viral isolates in cell cultures incubated for at least 28 days were positive by the rapid pre-CPE test (Table 4). Of the 29 pre-CPE-positive specimens, 12 were confirmed by another immunologic CMV reagent. In addition, previous or subsequent specimens from eight patients also yielded the virus in tube culture; one was from a newborn with a clinical diagnosis of a congenital viral disease, and one was a urine specimen from a patient with acquired immunodeficiency syndrome. Thus, 22 of the 29 discrepant results (76%) were very likely due to CMV in patients shedding the virus that did not produce CPE within 28 days.

In 14 instances, specimens yielded CMV in cell culture but not by the rapid monoclonal antibody shell vial assay. However, infected cells prepared from the conventional tube cell culture from two of these specimens were not confirmed immunologically by specific anti-CMV reagents. Overall, the results of these data are consistent with other large studies that have found the shell vial assay pre-CPE test to provide rapid, sensitive, and accurate results (6, 8, 10–12, 14, 17). Optimally, both methods should be used for maximum detection of CMV infection (11).

TABLE 3. Confirmation of CMV-infected cells from conventional tube cell cultures with the Syva CMV reagent

Trial site	No. (%) of positive results by:	
	CPE	Pre-CPE reagent with infected cells
1	41	40[a] (100)
2	25	25 (100)
3	43	36[b] (92)
4	46	39[c] (91)

[a] One CPE-positive culture was not tested with the pre-CPE reagent.
[b] Four CPE-positive cultures were not tested with the pre-CPE reagent.
[c] Three CPE-positive cultures were not tested with the pre-CPE reagent.

TABLE 4. Detection of CMV by pre-CPE reagent in shell vial and by CPE in conventional tube cell cultures

Trial site	No. of specimens with the results[a]:				Sensitivity (%)	Specificity (%)
	CT+/ SV+	CT+/ SV−	CT−/ SV+	CT−/ SV−		
1	40	1	3	159	98	98
2	23	2	6	89	92	94
3	36	7	7	344	84	98
4	42	4	13	161	91	93
All sites combined (total no. of specimens = 937)	141	14	29	753	91	96

[a] CMV-positive (+) and -negative (−) results obtained with the conventional tube (CT) culture and shell vial (SV) assays.

Incorporation of the monoclonal antibody directed to the immediate-early antigen of CMV provided the capability for detecting these infections as soon as 16 h postinoculation of the shell vial cell culture. Interestingly, two of the sites detected 69 of 71 (97%) CMV strains 16 to 24 h postinoculation, in contrast to the other two sites, which detected 68 of 95 (72%) (Table 2). Study sites 1 and 2 seeded shell vial cell cultures with trypsinized monolayer cultures prepared in their own laboratories, so that the cultures were inoculated with specimens within a 3- to 8-day time period after preparation. In contrast, sites 3 and 4 obtained from commercial sources shell vial cell cultures containing preformed monolayers that were at least 8 to 15 days old prior to inoculation with specimens. Thiele et al., using stock CMV pools, demonstrated that 4- to 11-day-old cell culture monolayers were more susceptible to viral infectivity than those inoculated 11 days after their preparation (18). This group speculated that cells may either lose receptors for maximal adsorption of the virus or may decline in cellular metabolism and division.

Only 55 of 71 (78%) CMV strains were detected by sites 1 and 2 (laboratory seeded) in the pre-CPE test at 40 to 48 h (Table 3). In general, cell culture monolayers examined at this second time period at sites 1 and 2 were more fragile and less intact than those stained at 16 to 24 h postinoculation. Perhaps the additional contact time of the specimen with the monolayer cells contributed to their toxicity, similar to that associated with conventional tube cell cultures that must be incubated for several days or weeks.

Evaluation of the Syva MicroTrak CMV culture identification test by four independent laboratories has indicated that the product is sensitive and specific for the rapid detection of CMV by the shell vial assay and reliably confirms the presence of the virus as detected by CPE in conventional tube cell cultures.

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