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Detection of Cytomegalovirus Antibody with Two Commercially Available Assays, an Indirect Hemagglutination Test and an Enzyme Immunosorbent Assay

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By the use of two reference procedures, an indirect hemagglutination assay and a complement fixation test, the presence or absence of cytomegalovirus (CMV) antibody was determined for 221 human sera. Ninety-nine sera (44.8%) were found to contain CMV antibody. The remaining 122 sera (55.2%) lacked detectable CMV antibody. These same sera were then analyzed by two recently introduced, commercially available CMV antibody assays, an indirect hemagglutination test (IHA-c; Cetus Corp., Emeryville, Calif.) and an enzyme-linked immunosorbent assay (ELISA; M. A. Bioproducts, Walkersville, Md.). With the results of the reference procedures as true evidence of the presence or absence of CMV antibody, the sensitivity of the IHA-c was found to be 100%; the specificity was 98.4%. The sensitivity of the ELISA was also 100%; the specificity was 96.7%. The overall accuracies of these procedures were 99.1 and 98.2%, respectively. Time and motion studies revealed the IHA-c procedure to be faster and technically less demanding than the ELISA procedure.

A rapid, convenient, accurate test for detecting cytomegalovirus (CMV) antibody would be of great value to clinical laboratories. Such a test is a prerequisite for the provision of CMV antibody-negative blood for transfusion in certain high-risk populations, such as neonates (15), organ transplant recipients (1, 7, 9), and possibly leukemic children not previously exposed to CMV. It might also be used to select antibody-negative organ donors for antibody-negative transplant recipients (10). Diagnostically, the absence of antibody to CMV can be used to exclude the possibility of congenital CMV infection, although its presence may reflect only transferred maternal antibody and not infection of the infant (11).

A variety of serological tests for CMV antibody have been devised, including complement fixation (CF), indirect or anticomplement immunofluorescent-antibody techniques, and indirect hemagglutination (IHA) procedures. The results of CF tests are highly dependent on the quality of CF antigen used. Because of this, false-negative CF results have been noted (4), and CF titers have been shown to vary over time in individuals known to have antibody, occasionally becoming undetectable (12). For these reasons, as well as for the practical difficulties centering around quality control of erythrocyte and complement reagents, CF is not suitable as a routine clinical laboratory procedure. Immunofluorescence may require tissue culture and fluorescence microscopy, which necessitates substantial interpretive skill and is therefore not feasible for many diagnostic laboratories. IHA is very reliable (14), but production of CMV antigen and sensitizing erythrocytes is not within the capabilities of most clinical laboratories. For these reasons, we chose to evaluate two recently introduced, commercially available CMV antibody tests, an IHA test (IHA-c; Cetus Corp., Emeryville, Calif.) and an enzyme-linked immunosorbent assay (ELISA; M. A. Bioproducts, Walkersville, Md.), for their accuracy and convenience in routine diagnostic laboratory use. Both of these procedures were shown recently to be highly accurate (8); however, comparisons were based largely on the results of other commercially available tests, and the study was restricted to specimens obtained from blood donors. In the present study, sera were obtained from nursing personnel and renal transplant recipients, and comparisons were made with results obtained in a reference laboratory (Centers for Disease Control, Atlanta, Ga.) by both a conventional IHA and a CF test.

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MATERIALS AND METHODS

Serum specimens. A total of 221 different sera were analyzed. Some serum specimens (120) were obtained randomly from 120 different healthy nurses working in intensive care units at a general, tertiary-care hospital for children. The remaining 101 sera were obtained from renal transplant patients after surgery. Serum specimens were stored at −65°C and thawed just before testing.

Reference procedures. Each of the 221 different sera was tested at the Centers for Disease Control for the presence of CMV antibody by both conventional IHA and CF tests (13). The IHA test was performed with tanned sheep erythrocytes sensitized with CMV antigen prepared from human embryonic lung fibroblasts infected with strain AD169 of CMV. Cells demonstrating maximum cytopathic effect for 24 h were dislodged with a rubber policeman, frozen and thawed in phosphate-buffered saline, and centrifuged to remove particulate cellular debris, and the supernatant was used to coat sheep erythrocytes. The reciprocal of the highest serum dilution which produced complete or almost complete agglutination was considered the IHA titer. The CF test was conducted with CMV antigen derived from infected cells that were dislodged in glycine-buffered saline (0.05 M glycine, pH 9.0), frozen and thawed once, disrupted by sonication, and clarified by centrifugation. The CF titer was taken as the reciprocal of the highest dilution of serum yielding 70% CF as judged by the degree of hemolysis.

IHA-c. All sera were analyzed by a qualitative, commercially available IHA test (IHA-c) according to the instructions of the manufacturer (Cetus). Briefly, 0.1 ml of undilut-

ed test serum was mixed with 0.3 ml of buffered salts solution in normal rabbit serum and heated to 56°C for 30 min. A fifty-μl volume of this mixture was transferred to a U-bottomed well in a microtiter plate and mixed with 25 μl of a suspension containing CMV antigen-sensitized human type O erythrocytes. The plate was sealed and incubated at room temperature for 90 min. The presence of a discrete erythrocyte button with clearly distinguished margins was interpreted as a negative test. Any other pattern was interpreted as a positive test. Incubation of plates for as long as 24 h at room temperature did not change the test results. All test sera were examined for the presence of nonspecific reactivity by mixing with unsensitized erythrocytes. In addition, known positive and negative control sera were included with each test run. All sera were tested independently by two technologists without knowledge of the results of reference procedures. Both technologists had extensive experience with the IHA-c procedure. When discrepancies were noted between the results obtained by the two technologists, the tests were repeated.

Commercial ELISA. All sera were tested by a commercially available ELISA, according to the instructions of the manufacturer (M. A. Bioproducts). Briefly, plastic U-bottomed microtiter wells coated with CMV antigen were washed three times with a phosphate-buffered saline solution containing Tween 80. Undiluted human serum diluent (250 μl) was mixed with 10 μl of test serum in individual wells and incubated for 45 min at room temperature. The test serum mixture was removed, and 250 μl of alkaline phosphatase-conjugated rabbit antihuman immunoglobulin G was added to each well. After incubation for 45 min at room temperature, the conjugate was removed and replaced with 250 μl of *para*-nitrophenyl phosphate enzyme substrate. After 45 min at room temperature, the reaction was stopped by the addition of 50 μl of 1 N NaOH. Substrate conversion was measured with an automated micro-ELISA reader (Minireader II, Dynatech Laboratories, Inc., Alexandria, Va.). Results were machine interpreted as negative, equivocal, low positive, medium positive, or high positive, with at least three categories representing evidence of CMV antibody in the test serum. All test sera were examined for the presence of nonspecific reactivity with wells coated with uninfected cells. Known negative, low-positive, and high-positive control sera were included with each test run. All sera were tested independently by two technologists without knowledge of the results of the reference procedures or of the IHA-c. Both technologists had extensive experience with the ELISA procedure. When discrepancies were noted between the results obtained by the two technologists, the tests were repeated.

Statistical analyses. Calculations of test accuracy, sensitivity, specificity, predictive value of a positive test result, and predictive value of a negative test result were made by methods described by Galen and Gambino (5).

RESULTS

Among the 221 sera tested by the reference CF and IHA procedures, 122 (55.2%) sera were found to have titers of <8 by both procedures and thus were considered negative for CMV antibody. The remaining 99 sera (44.8%) were found to have titers of ≥8 by both tests. These sera were considered positive for CMV antibody. Comparison of the actual titers obtained by CF with those obtained by IHA revealed no obvious quantitative relationship between the titers obtained by these two procedures.

When the 221 sera were tested by the IHA-c, complete agreement was observed between the results of tests conducted independently by two technologists with 218 (98.6%) samples. In three cases, positive results were obtained by one technologist and negative results were obtained by the other. Retesting resolved two of the discrepancies. In one case, however, retesting consistently yielded negative results by one technologist and positive results by the other. For purposes of comparison with the results of the reference procedures, this specimen was considered equivocal by the IHA-c. Nine discrepancies between the results obtained by two technologists were noted with the ELISA. In three instances, negative results were obtained by one technologist and equivocal results were obtained by the other. In six cases, one technologist obtained positive results, whereas the other obtained equivocal results. One repeat testing resolved eight of these discrepancies. In a single specimen, repetitive testing by one technologist consistently yielded negative results, whereas repetitive testing by the other technologist consistently yielded equivocal results. For purposes of comparing the CMV ELISA with the reference procedures, this serum was considered equivocal.

A comparison of the results obtained by the IHA-c and the ELISA with those obtained by the reference procedure (i.e., CF and conventional IHA) is seen in Table 1. Concordance between the results of the IHA-c and the ELISA was observed with all 99 sera determined to be positive for CMV antibody by the reference procedures. Of the 122 sera (98.4%) found by the reference tests to lack CMV antibody, 120 were also negative by the IHA-c. One of the two sera in which discordance was noted yielded equivocal results by the IHA-c (described above); the other was determined to be positive by this method. This specimen was also positive by the ELISA. Four discordant results were obtained with the ELISA among the 122 found to lack CMV antibody by the reference procedures. In three cases, positive results were obtained with the ELISA. In one case (described above), equivocal results were obtained.

The overall accuracies of the IHA-c and the ELISA were 99.1 and 98.2%, respectively. The sensitivity of both the IHA-c and the ELISA was 100%. The specificities of the IHA-c and the ELISA were 98.4 and 96.7%, respectively. Assuming a prevalence of CMV antibody of 40 to 70% in the general population (2, 3, 6), the predictive value of a positive test result with the IHA-c used as a screening procedure would be 97.6%; the predictive value of a negative test result would be 100%. The predictive value of a positive test result for the ELISA would be 95.2%; the predictive value of a negative test result would be 100%.

Time and motion studies were conducted with both procedures on the basis of testing 5, 10, and 30 samples. Thirteen individual manipulations were identified by the IHA-c procedure. The hands-on technologist times required for these manipulations were 38, 51, and 106 min for 5, 10, and 30 specimens, respectively. Regardless of the sample number, a 90-min incubation period was also required. Therefore, the total times required to achieve a test result with the IHA-c would be 128, 141, and 196 min, respectively, for 5, 10, and 30 specimens.

Twenty-one different steps were defined with the CMV ELISA procedure. For testing 5, 10, and 30 specimens, the hands-on technologist times were determined to be 120, 139, and 177 min, respectively. These determinations were predicated on the use of an automated microtiter reader both for interpreting reactions and for calculating test results. The ELISA necessitated three 45-min incubation periods. The total times required to achieve a test result with this system,

TABLE 1. Comparison of the results of two commercial CMV antibody tests, IHA-c and ELISA, with the results of reference procedures for 221 sera[a]

Presence of CMV antibody in reference tests	No. of sera	No. (%) of sera yielding concordant results by:	
		IHA-c	ELISA
Positive	99	99 (100)	99 (100)
Negative	122	120 (98.4)	118 (96.7)

[a] Reference procedures employed were a CF test and an IHA test. Complete agreement was obtained between the results of these two procedures for all 221 sera analyzed.

assuming a test volume of 5, 10, and 30 specimens, would therefore be 244, 274, and 312 min, respectively.

DISCUSSION

After discrepancies between the results obtained by two technologists were resolved, both the IHA-c and the ELISA were found to be highly accurate. Both demonstrated 100% agreement with reference methods when applied to sera containing CMV antibody. Concordance was achieved with 98.4 and 96.7% of CMV antibody-negative sera, respectively. These results are consistent with findings obtained in a previous study (8). Since a principal clinical utility of this type of CMV serological test would be to demonstrate the absence of CMV antibody in sera of organ transplant donors and neonates suspected of having congenital CMV infection, the reliability of a seronegative result was of particular interest. Since both the IHA-c and the ELISA were 100% sensitive, i.e., no false-negative results were obtained, the predictive value of a negative test result with these assays was also 100%. It follows, therefore, that both procedures could be used with confidence for screening sera for these applications. It should be emphasized that the calculations of predictive value performed in this study should be applicable to general laboratory practice, since the prevalence of CMV antibody in our sample (i.e., 44.8%) closely approximates that reported for the general population (i.e., 40 to 70%) (2, 3, 6).

In comparing the two methods, however, the number of discrepancies observed between the results obtained independently on the same sera by two technologists become an important consideration. These discrepancies were apparently the result of variation inherent in the test methodologies rather than in lot-to-lot variation in assay reagents, since test kits from the same lot were used by both technologists when testing the same sera. Discrepancies were noted with 3 of 221 sera (1.4%) with the IHA-c and with 9 of 221 sera (4.1%) with the ELISA. These discrepancies mean that one technologist obtained an incorrect result. Had the result of the other technologist not been available, the total number of discordant results listed in Table 1 might have been higher. It would seem prudent, therefore, at least with the ELISA, to analyze clinical specimens in duplicate.

The total hands-on technologist time required to perform the IHA-c was considerably less than that required for the ELISA, although the time increment required for each additional specimen was smaller with the ELISA. Not included in estimates of hands-on technologist time was the length of requisite incubation periods. In the case of ELISA, this omission is probably misleading, since the incubation period (three for 45 min each) was relatively short and needed to be carefully timed, thus obviating the possibility of technologists performing other, unrelated tasks during these intervals.

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