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Comparison and Correlation of Four Methods of Measuring Immunity to Human Cytomegalovirus

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Abstract

COMPARISON AND CORRELATION OF FOUR METHODS OF MEASURING IMMUNITY TO HUMAN CYTOMEGALOVIRUS

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The complement fixation test (CF) is currently the test most often used to determine the presence of antibody to human Cytomegalovirus (CMV). Several more recent techniques have now been adapted for this purpose. A comparison of CMV antibody titers was made between the CF test, a commercially available enzyme-linked immunosorbent assay (ELISA), an indirect immunofluorescent technique (IFA), and a modified indirect hemagglutination test (IHA). Forty three serum samples were tested for antibodies to CMV by each of the above procedures. The ELISA, IFA, and IHA titers were in close agreement on all samples tested. The ELISA, IFA, and IHA titers were all equal to or greater than the CF titer for 38 of the 43 samples (88%). Two samples were anticomplementary in the CF test but gave readable results in the other procedures. Two samples with low CF titers were negative in each of the other tests. The CF test was the least sensitive of the procedures examined. The commercial ELISA system appeared to be the most practical method and offered the highest degree of sensitivity in detecting antibodies to CMV.

LOMA LINDA UNIVERSITY

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COMPARISON AND CORRELATION OF FOUR METHODS OF MEASURING IMMUNITY TO HUMAN CYTOMEGALOVIRUS

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Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.

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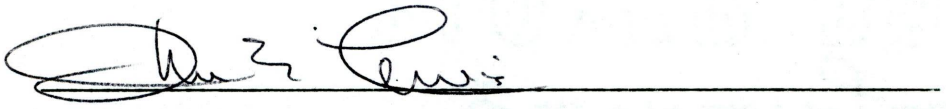

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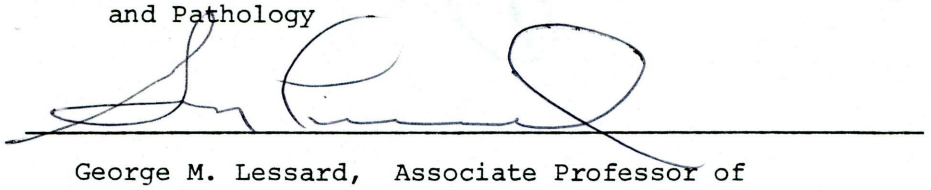

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It is now recognized that Cytomegalovirus (CMV) infection can be acquired as a result of blood transfusion. The possibility of transmitting CMV via blood donors with latent CMV infection was suggested as early as 1951 (1). Subsequently, an infectious mononucleosis-like illness identified as a postperfusion syndrome was shown to be caused by CMV (2,3). Convincing evidence for blood as a source of CMV has been reported since that time (4).

More recent studies have shown that up to 25% of exchange-transfused newborns may develop CMV infections (5). In one study, 13.5% of infants of CMV seronegative mothers developed infection when exposed to at least one donor with serologic evidence of latent CMV infection. Fatal or serious symptoms developed in 50% of these infected infants who had received more than 50 ml of packed red blood cells. It was shown that the use of CMV seronegative donors virtually eliminated transfusion-acquired CMV infections in this group of subjects (6).

CMV appears to be associated with leukocyte-rich fractions of fresh whole blood in various reports (7,8,9). Evidence also indicated that the granulocyte fraction is more closely associated with CMV than is the mononuclear fraction (8). Complicating the picture is the additional finding that CMV has been recovered from the erythrocyte, plasma, and serum fractions of blood as well as from cerebrospinal fluid (10,11). The solutions offered to the problem of transfusion-acquired CMV infection have focused primarily on the use of leukocyte-poor blood or the use of donors who have no serologic evidence of antibodies to CMV. Of these

approaches to the problem, the use of CMV seronegative blood appears in most cases to be the most practical procedure.

The intent of this study was to evaluate and compare several current methods for detecting the presence of antibody to CMV. The overall purpose was to determine the most practical method for clinical laboratories to screen blood used to transfuse certain "high risk" patients. The complement fixation test (CF) was chosen as the standard for comparison because of it's longstanding and widespread use. It is the most commonly used test for detecting the presence of antibody to CMV. The indirect fluorescent antibody technique (IFA), a modified indirect hemagglutination test (IHA), and a commercially available enzyme-linked immunosorbent assay (ELISA) were chosen as examples of more recent techniques that are currently in use and may offer certain advantages over the CF test.

The data demonstrate that for the majority of samples tested each method gave approximately the same qualitative result, either positive or negative for the presence of antibody to CMV. The CF test appeared less sensitive than the other methods examined and consistently gave lower titers. The IFA, IHA, and ELISA assays were in close agreement with each other. The commercial ELISA technique was shown to be the most sensitive procedure by demonstrating the highest antibody titers to CMV and in addition, appeared to be the easiest to set up and interpret.

MATERIALS AND METHODS

Sera Selection

Sera used were selected from those submitted to the diagnostic

virology laboratory at Loma Linda University Medical Center for determination of antibody titers to CMV. All sera had been previously tested for CMV antibody by the CF method and were stored frozen at -20°C. Sera were selected to include samples showing no anti-CMV activity as well as samples representing low, medium, and high titers to CMV. Ten of the samples represented paired sera (acute and convalescent) and thirty three were single serum samples.

Complement Fixation Assay

CF tests were done by a standard microtiter method (12) using 2 units of antigen. Antigen standardization was accomplished by testing serial twofold dilutions of immune serum to determine the optimal dilution of antigen which gave fixation. A commercial antigen prepared from CMV strain AD-169 was used (Flow Laboratories, Inglewood, Calif. Lot #33015). Guinea pig complement (Lot #31009), hemolysin (rabbit anti-sheep erythrocyte serum Lot # 4788), CMV antigen control (Lot #33471), and sheep red blood cells (Lot #A4892) were also obtained from the same manufacturer. Serum titers were expressed as the reciprocal of the highest dilution in which 70% or more of the sheep red blood cells are not hemolysed. No hemolysis indicates a positive test.

Enzyme-Linked Immunosorbent Assay

Detection of antibody by the CMV BIO-BEAD TITRATION KIT (Litton Bionetics, Kensington, Md.) is based on the principle of solid phase immunoassay. A magnetic transfer device, available from the same manufacturer, is used to transfer the solid phase from one reaction mixture to another.

All reagents, controls, and equipment for performing the test were supplied by the manufacturer.

Ferrous metallic beads with bound CMV antigen from infected cell cultures were used in the test. The beads were placed in the dilution of serum to be assayed and specific antibodies, if present, would attach themselves to the antigens bound to the beads. The beads were washed to remove unbound human serum proteins and then released into a reaction mixture containing peroxidase conjugated to goat anti-human immunoglobulins. The conjugate attached to the patient's antibody if the patient's antibody was attached to the CMV antigen on the beads. The beads were then washed to remove unbound conjugate and placed into a substrate solution (0.03% 2,2'-Azino-di-3-ethyl-benzthiazoline-sulfonate-6). The appearance of a green color indicated the presence of human antibody to the antigens on the beads.

The antibody titer was determined as the highest dilution of serum in which a 1+ to 2+ green color developed with the antigen coated bead. The degree of color development was compared to that yielded by the positive control sera at the stated titers. A definite but light green color was considered to be a 1+ to 2+ reaction and a dark green color was read as a 3+ to 4+ reaction. For the purposes of this study, the procedure as outlined by the manufacturer (package insert; CMV BIO-BEAD TITRATION KIT, Litton Bionetics, Kensington, Md.) was followed with the exception that the initial serum dilution was 1:8, not 1:100 as specified in the package protocol.

Indirect Fluorescent-Antibody Assay

For the IFA test, the AD-169 strain of CMV was cultured on L-645 human lung fibroblasts until 70% cytopathic effect was noted (approximately 120 hrs. post infection). The cells were then dislodged with 0.25% trypsin, washed twice with phosphate-buffered saline (PBS; 0.15M, pH 7.2) and adjusted to a final concentration of approximately 10^7 cells per ml. One drop of cell suspension was placed on circumscribed areas of glass slides and air dried. The air dried slides were then fixed with cold acetone for 15 minutes at 4°C and stored frozen at -70°C until needed for the test itself.

Sera were diluted in PBS and serial twofold dilutions were prepared from 1:8 through 1:256. Each dilution was individually applied dropwise to a focus of dried infected cells and the slides placed in a moist chamber for 20 minutes at 37°C. The preparations were washed twice with PBS and then overlaid with a drop of goat fluorescein-conjugated anti-human immunoglobulin G (Meloy Laboratories, Springfield, Va. Lot #78077) for 20 minutes at 37°C. (Conjugates were first titrated against previously defined known positive serum to determine the optimal dilution for use.) The conjugate was removed, the cells were washed in PBS as before, and the preparations were covered with 25% glycerin in PBS at pH 9. Positive and negative serum controls were included on each slide.

A positive reaction was indicated by specific nuclear fluorescence observed by microscopic examination with ultraviolet light. The antibody

level was expressed as the dilution or titer which maintained a 1+ fluorescence as determined by the reader. The IFA test as performed in this study was a well standardized IFA procedure commonly used to detect the presence of antibody to CMV (15).

Indirect Hemagglutination Assay

The IHA procedure was performed as outlined by Yeager (13). This procedure differed from earlier IHA tests for CMV (14) in that glutaraldehyde-fixed, human type O erythrocytes rather than sheep erythrocytes were used. This method offered the advantage of freezing glutaraldehyde-fixed, tanned, sensitized cells for long periods until needed for the test. The procedure was carried out in several steps. Freshly drawn whole human blood from a donor with type O erythrocytes was mixed with sodium citrate, washed several times in PBS and fixed in 1% glutaraldehyde (EM grade, Sigma Chemical Co. St. Louis, Mo.) for 30 minutes. After fixation, the cells were again washed and could be used immediately or stored frozen at -70°C for up to six months. The antigen used was prepared from CMV strain AD-169 grown in human foreskin fibroblasts. Yeager's procedure specified a freeze-thaw extraction technique. We extracted the antigen by sonic treatment of the infected cell suspension after a cycle of freezing and thawing.

The optimal dilution of antigen was determined by titrating the antigen against known positive sera using cells treated with the optimal tannic acid concentration for each cell batch. The optimal antigen dilution was 1:8 in our preparation. The optimal tannic acid concentration was determined for each cell batch by testing known positive and negative sera

against cells with tannic acid concentrations in the range of 1:20,000 to 1:320,000 (wt/vol) and sensitized with the optimal dilution of antigen. The optimal tannic acid concentration in our test system was 1:40,000. Once the correct antigen and tannic acid concentrations were determined, the fixed, tanned, sensitized human O erythrocytes and control cells (cells treated in like manner but not sensitized with CMV antigen) were stored frozen until needed for the test. The test itself was easily performed.

The cells were added to serial twofold dilutions of serum in 0.15M PBS (pH 7.2) containing 1% normal rabbit serum and .1M lysine. The serum dilutions ranged from 1:8 to 1:1024. The cells were allowed to settle for forty five minutes at room temperature and read. The titer was considered to be the highest dilution of serum that caused a 3+ to 4+ agglutination.

RESULTS

The results, summarized in Table 1, are expressed as the reciprocal of the dilutions obtained as titers in the various tests. The sera are arranged according to increasing CF titers since the CF test served as our baseline data against which all other procedures were compared.

Two samples (#42 and #411) gave low CF titers but were negative in each of the other three test systems. Two samples (#777 and #1231) were anticomplementary (AC) in the CF test even after several attempts to eliminate the anticomplementary activity. It is interesting to note that one of the AC samples was measured as a high-titered positive in the other test procedures and the other AC sample was shown to be negative.

With the exception of these four samples, all sera that were positive in one test system were also positive in each of the other tests. Conversly, all sera giving negative results in one test, gave the same result in each of the other methods. The results given by all tests on these sera were qualitatively the same.

The titers obtained demonstrated the quantitative differences between the four methods. The CF test generally gave lower titers than the other procedures examined. In Figure 1, the CF titers are compared directly with the titers obtained by the ELISA, IFA, and IHA assays. These data demonstrate that each of these procedures offered a higher degree of sensitivity than did the CF test as evidenced by the consistently higher titers obtained. The AC sera were excluded from Figure 1. The IHA titer was carried out to a 1:1024 dilution as specified by the procedure while all other methods were carried out only to a 1:256 dilution.

Figure 2 shows a comparison of titers obtained by the ELISA, IFA, and IHA tests. It can be seen that the titers obtained by these three methods demonstrated a high degree of correlation. This correlation was analysed statistically using Spearman's rank correlation method and the results are presented in Table 2. The high correlation coefficients (r) obtained suggest that the four procedures are measuring similar antibody to CMV. The higher degree of correlation between the ELISA, IFA, and IHA results (r values above .8) are in contrast to the lower correlation coefficients obtained when comparing the CF test results with the other procedures (r values between .4 and .6). Reliable coefficients of correlation ranging from .4 to

.6 may be regarded as indicating a moderate degree of correlation while values from .8 to 1.0 indicate a high correlation (16).

Figure 3 shows the distributions of the titers obtained by each method. It is evident that the titers from the ELISA, IFA, and IHA assays are more sensitive than the titers from the CF test. These differences are reflected in the geometric mean titers (GMTs) calculated for each test. The GMTs for the ELISA, IFA, and IHA tests do not differ significantly from one another but they are all significantly greater than the GMT calculated for the CF test. For the sake of comparison, all titers greater than 1:256 in the IHA test are grouped in the 1:256 category.

DISCUSSION

It has been estimated that 66% of the general population of the United States shows serologic evidence of CMV infection (17) and 12% of all blood donors are capable of transmitting the virus (18). In a routine sampling of 1000 units of blood, 21% had significant titers (1:8 or greater) of CF antibody to CMV (19). The presence of antibody to CMV does not necessarily mean that one is a carrier of the virus (20) but there is a definite positive correlation between transfusion-acquired CMV infection and the presence of antibody. The amount of blood received is also a factor in establishing CMV infection via blood donors (6,21).

The CF test, although widely used, may have several limitations as a diagnostic procedure. The sensitivity of the assay is uncertain and small quantities of antibody may not be detected (22,23). The CF test primarily detects the presence of specific IgG antibodies. Not all subclasses

of IgG fix complement (24) and IgM apparently reacts poorly in the CF test as used for CMV (25). A negative CF test does not rule out CMV infection and its usefulness in screening prospective blood donors is limited.

Our results support the conclusions of other investigators that the CF test lacks sensitivity (26,29). The CF titers were consistently lower than the titers achieved with the other methods. All of the sera that were positive in the ELISA, IFA, and IHA tests were also positive in the CF test with the exception of the two AC samples. From this standpoint, the CF test was adequate in detecting CMV antibody but did so at a lower level of sensitivity. The AC samples demonstrated another problem with the CF procedure in that not all sera give acceptable results in the test. Anticomplementary sera are those reading less than 75% hemolysis in the serum control without antigen. Samples #42 and #411 resulted in CF titers of 1:8 and 1:16 respectively, but were negative in all other assays examined. The CF testing was done prior to the other procedures. It is possible that additional freezing and thawing of these samples adversely affected the ELISA, IFA, and IHA results. A repeat CF assay was not performed on these two samples due to insufficient sample. It was noted that there was an occasional decrease in titer when certain sera had undergone repeated cycles of freezing and thawing.

It is also possible that the CF test may be detecting specific antibodies that are somehow different from those detected by other assays (26). It has been proposed that "early" antibody from recent CMV infections may result in different antigen binding properties from those usually encountered,

or that these types of results may reflect differences in the antigens of the test systems (14). The sensitivity of the test may be increased by using a glycine extraction technique for the antigen preparation. The glycine-extracted antigen may result in higher CF titers and CMV antibodies may be detected more frequently (27).

The ELISA technique has been adapted to a variety of test systems for measuring antibody to CMV. The flexibility of the assay has been adequately demonstrated by one investigator who evaluated seven different ELISA assays for antibody to CMV (29). The ELISA technique has been shown to be effective in detecting and titrating CMV antibody (30). Both IgG (30) and IgM (30,31) antibodies to CMV have been demonstrated by this procedure. The sensitivity of the test is reported to be high and the titers obtained compare favorably with both the IFA (30) and the IHA (32) procedures.

In the present study, the ELISA was found to be easily performed. All controls, reagents, and test materials were available from the manufacturer (Litton Bionetics, Kensington, Md.) in kit form. As was shown in the results, the ELISA demonstrated the highest degree of sensitivity in comparison with the other methods examined. The ELISA test was the most practical procedure based on ease of handling, speed (less than 4 hrs.), and interpretation of results. In order to make titer comparisons with all four methods, sera were diluted 1:8 through 1:256. This was contrary to the manufacturer's recommendation to use dilutions from 1:100 through 1:6400. The lower dilutions presented no difficulty in interpretation.

Two samples (#234 and #188) gave ELISA titers less than 1:100. The manufacturer's claim that a 1:8 CF titer is equal to a 1:100 ELISA titer was not supported by these results.

The IFA test is reported to be more sensitive than the CF test (33,34) and can be used to effectively screen blood for antibody to CMV (5). CMV infection of human fibroblasts induces an Fc receptor in the cytoplasm of infected cells which may non-specifically bind IgG. This can result in false positive reactions with certain samples (15). Non-specific staining can be minimized by using isolated nuclei for target antigens (35) or the test can be performed as an anticomplement immunofluorescence procedure (36).

The IFA procedure compares well with the IHA test and may be more sensitive (39). It can also be used to measure antibody classes other than IgG (37,38). Our results indicated a high degree of correlation with both the ELISA and IHA procedures (Figure 2). Training individuals to competently read the IFA test may be time consuming. With experience, one can learn to correctly distinguish between specific nuclear staining and non-specific cytoplasmic staining. The IFA test is rapid (less than 2 hrs.) and convenient to use once the slides have been prepared.

The IHA test is also a highly sensitive and reproducible procedure. The test is more sensitive than the CF test (14,40) and IgM antibodies to CMV can be assayed for as well as IgG. The IHA system correlates well with the IFA and ELISA procedures. It has an additional advantage in that IHA antibodies are some of the first to appear in a CMV infection (41).

The IHA titers being higher than the CF titers may be due to fundamental differences in the antigens used in the tests (42). The modified IHA procedure that we used (13) utilizes human type O erythrocytes and permits storage of fixed, tanned, sensitized cells frozen for up to eight months. Long term storage of CMV sensitized sheep cells has been one of the major limitations of the standard IHA procedure. Recently, the use of freeze-dried sensitized cells has been perfected and is being advocated (43).

The IHA test itself is a rapid procedure (45 minutes) and the test iseasily performed. The test can be used effectively to screen large numbers of sera for CMV. The preparation and pH of the various buffers were found to be critical for proper results. The tannic acid titration was an exacting procedure and not all of our human O cell batches would work in the test (4 out of 7). Each batch of cells reacted differently and had to be titrated seperately. It is also of interest that glycine-extracted antigen failed to perform satisfactorily in the IHA procedure. It was due to these types of problems that the test was not considered to be a practical method in our laboratory.

In conclusion, the CF test was found to be the least sensitive method used but it's effectiveness may still be valid as there were two samples that were positive by no other procedure. The ELISA was shown to be the most sensitive test and was also the easiest to use. The results of the IFA and IHA tests were comparable to the ELISA but these procedures were shown to be less desirable from a practical standpoint. As a result of this study the virology laboratory at Loma Linda University Medical Center

will be using the ELISA technique when assaying sera for antibodies to CMV.

LITERATURE CITED

1. Wyatt, J.P., F.A. Hemsath, and M.D. Soash. 1951. Disseminated Cytomegalic Inclusion Disease In An Adult With Primary Refactory Anemia and Transfusional Siderosis. Amer. J. Clin. Pathol. 21:50-55.

2. Kaarianen, L., E. Klemola, and J. Paloheimo. 1966. Rise Of Cytomegalovirus Antibodies In An Infectious Mononucleosis-Like Syndrome After Transfusion. Br. Med. J. 1:1270-1272.

3. Kantor, G.L., and L.S. Goldberg. 1971. Cytomegalovirus Infections After Open Heart Surgery. Lancet. 1:777-780.

4. Diosi, P., E. Moldovan, and N. Tomescu. 1969. Latent Cytomegalovirus Infection In Blood Donors. Br. Med. J. 4:660-662.

5. Benson, J.W.T., S.J. Bodden and J. Tobin. 1979. Cytomegalovirus and Blood Transfusion In Neonates. Arch. Dis. Child. 54:538-541.

6. Yeager, A.S., F.C. Grumet, E.B. Hafleigh, A.M. Arvin, J.S. Bradley, and C.G. Prober. 1981. Prevention of Transfusion-Acquired Cytomegalovirus Infections In Newborn Infants. J. Pediatr. 98:281-287.

7. Lang, D.J. 1977. Reduction of Postperfusion Cytomegalovirus Infections Following The Use of Leukocyte Depleted Blood. Transfusion. 17:391-395.

8. Howell, C.L., M.J. Miller, and W.J. Martin. 1979. Comparison of Rates of Virus Isolation and Ficoll-Paque/Marodex Methods. J. Clin. Microbiol. 10:533-537.

9. Winston, D.J. 1980. Cytomegalovirus Infections Associated With Leukocyte Transfusions. Ann. Intern. Med. 93:671-675.

10. Armstrong, D., M. Ely, and L. Steger. 1971. Posttransfusion Cytomegaloviremia and Persistence of Cytomegalovirus In Blood. Infect. Immunol. 3:159.

11. Torrey, E.F., R.H. Yolken, and C.J. Winfrey. 1982. Cytomegalovirus Antibody in Cerebrospinal Fluid of Schizophrenic Patients Detected by Enzyme Immunoassay. Science. 216:892-894.

12. Lennette, E.H., E. Spaulding, and J. Truant (ed.). 1974. Manual of Clinical Microbiology, Second ed. pp. 667-837.

13. Yeager, A.S. 1979. Improved Indirect Hemagglutination Test For Cytomegalovirus Using Human O Erythrocytes In Lysine. J. Clin. Microbiol. 10:64-68.

14. Bernstein, M.T., and J.A. Stewart. 1971. Indirect Hemagglutination Test For Detection of Antibodies to Cytomegalovirus. Appl. Microbiol. 21:84-89.

15. Rose, N.R., and H. Friedman (ed.). 1980. Manual of Clinical Immunology, Second ed. American Society For Microbiology. pp. 622-627.

16. Franzblau, A.H. 1958. A Primer of Statistics for Non-Statisticians. Harcourt, Brace and World Inc. New York. p. 81.

17. Tolkoff-Rubin, N.E. 1978. Cytomegalovirus Infection In Dialysis Patients and personnel. Ann. Intern. Med. 89:625-628.

18. Prince, A.M. 1971. A serologic Study of Cytomegalovirus Infections Associated With Blood Transfusion. N. Engl. J. Med. 284:1125.

19. Monif, G.R.G., W.R. Adams, and L.L. Flory. 1974. Complement Fixing Antibodies to the AD-169 Strain of Cytomegalovirus In Banked Blood. Transfusion. 14:58-60.

20. Monif, G.R.G., G.I. Daicoff, and L.L. Flory. 1976. Blood As A Potential Vehicle for the Cytomegalovirus. Amer. J. Obstet. Gynecol. 126: 445-448.

21. Stevens, D.P., L.F. Barker and A.S. Ketchaus. 1970. Asymptomatic Cytomegalovirus Infection Following Blood Transfusions In Tumor Surgery. J. Amer. Med. Assoc. 211:1341-1344.

22. Horodniceanu, F., and S. Michelson. 1980. Assessment of Human Cytomegalovirus Antibody Detection Techniques. Arch. Virol. 64:287-301.

23. Lang, D.J., and J.B. Hanshaw. 1969. Cytomegalovirus Infection and the Postperfusion Syndrome. Recognition of Primary Infections In Four Patients. N. Engl. J. Med. 280:1145-1149.

24. Ishizaka, T.F., F. Ishizaka, S. Salmon, and H. Fudenberg. 1967. Biologic Activities of Aggregated Gamma Globulins. J. Immunol. 99:89-91.

25. Lang, D.J., and B. Noren. 1968. Cytomegaloviremia Following Congenital Infection. J. Pediatr. 73:812-819.

26. Griffiths, P.D., K.J. Buie, and R.B. Heath. 1978. A Comparison of Complement Fixation, Indirect Immunofluorescence for Viral Late Antigens and Anti-Complement Immunofluorescence Tests for the Detection of Cytomegalovirus Specific Serum Antibodies. J. Clin. Pathol. 31: 827-831.

27. Cremer, N.E., N.J. Schmidt, F. Jensen, M. Hoffman, L.S. Oshiro, and E.H. Lennette. 1975. Complement-Fixing Antibody In Human Sera Reactive With Viral and Soluble Antigens of Cytomegalovirus. J. Clin. Microbiol. 1:262-267.

28. Yolken, R.H., and P.J. Stopa. 1980. Comparison of Seven Enzyme Immunoassay Systems For Measurement of Cytomegalovirus. J. Clin. Microbiol. 11:546-551.

29. Booth, J.C., G. Hannington, T.A.G. Aziz, and H. Stern. 1979. Comparison of Enzyme-Linked Immunosorbent Assay Technique and Complement Fixation Test For Estimation of Cytomegalovirus IgG Antibody. J. Clin. Pathol. 32:122-127.

30. Schmitz, H., H.W. Doerr, D. Kampa, and A. Vogt. 1977. Solid Phase Enzyme Immunoassay For Immunoglobulin M Antibodies to Cytomegalovirus. J. Clin. Microbiol. 5:629-634.

31. Yolken, R.H., and F.J. Leister. 1981. Enzyme Immunoassays For Measurement of Cytomegalovirus IgM Antibody. J. Clin. Microbiol. 14:427-432.

32. Castellano, G.A., G. Hazzard, D. Madden, and J. Severn. 1977. Comparison of the Enzyme-Linked Immunosorbent Assay and the Indirect Hemagglutination Test For Detection of Antibody to Cytomegalovirus. J. Infect. Dis. 136:s337-s340.

33. de Silva, L.M., F.L. Kampfner, C.M. Lister, and J.O'H. Tobin. 1977. Identification of Pregnancies At Risk From Cytomegalovirus Infection. J. Hygiene. 79:347-354.

34. Betts, R.F., S.D. George, B.B. Rundell, R.B. Freeman, and R.G. Douglas Jr. 1976. Comparative Activity of Immunofluorescent Antibody And Complement-Fixing Antibody In Cytomegalovirus Infection. J. Clin. Microbiol. 4:151-156.

35. Stagno, S.D., D.W. Reynolds, and R.J. Smith. 1978. Use of Isolated Nuclei In the Indirect Fluorescent Antibody Test For Human Cytomegalovirus Infection; Comparison with Microneutralization, Anticomplement, and Conventional Indirect Fluorescent Antibody Assays. J. Clin. Microbiol. 7:486-489.

36. Kettering, J.D., N.J. Schmidt, D. Gallo, and E.H. Lennette. 1977. Anticomplement Immunofluorescence Test For Antibodies To Human Cytomegalovirus. J. Clin. Microbiol. 6:627-632.

37. The, T.H., G. Klein, and M.M.A.C. Langenhuysen. 1974. Antibody Reactions To Virus Specific Early Antigens In Patients With Cytomegalovirus Infections. Clin. Exp. Immunol. 16:1-12.

38. Waner, J.L., D.R. Hopkins, T. Weller, and E.N. Alred. 1977. Cervical Excretion of Cytomegalovirus; Correlation With Secretory and Humoral Antibody. J. Infect. Dis. 136:805-809.

39. Stagno, S.D., D.W. Reynolds, A. Tsiantes, D.A. Fuccillo, W. Long, and C.A. Alford. 1975. Co,parative Serial and Virologic Studies of Symptomatic and Subclinical Congenitally and Neonatally Acquired Cytomegalovirus Infections. J. Infect. Dis. 132:568-577.

40. Cremer, N.E., M. Hoffman, and E.H. Lennette. 1978. Analysis of Antibody Assay Methods And Classes of Viral Antibodies In Serodiagnosis of Cytomegalovirus Infection. J. Clin. Microbiol. 8:153-159.

41. Michelson, S., N. Cabau, A. Boue, and F. Horodniceanu. 1979. Comparison of Occurance of Antibody To Human Cytomegalovirus As Demonstrated By Immunofluorescence And Indirect Hemagglutination. J. Clin. Microbiol. 9:149-151.

42. Benyesh-Melnick, M., V. Vonka, and F. Probstmeyer. 1966. Human Cytomegalovirus: Properties of the Complement-Fixing Antigen. J. Immunol. 96:261-267.

43. Cabau, N., R. Crainic, C. Davis, G. Denoyel, A. Gaspar, C. Bronnert, A. Boue, and F. Horodniceanu. 1981. Freeze-Dried Erythrocytes for An Indirect Hemagglutination Test For Determination of Cytomegalovirus Antibody. J. Clin. Microbiol. 13:1026-1o30.

TABLE 1 - SUMMARY OF TITERS OF FOUR METHODS OF MEASURING ANTIBODY TO CYTOMEGALOVIRUS

SAMPLE #	CF TITER	ELISA TITER	IFA TITER	IHA TITER
152	<8	<8	<8	<8
184	<8	<8	<8	<8
199	<8	<8	<8	<8
221	<8	<8	<8	<8
286	<8	<8	<8	<8
315	<8	<8	<8	<8
347	<8	<8	<8	<8
388	<8	<8	<8	<8
429	<8	<8	<8	<8
445	<8	<8	<8	<8
503	<8	<8	<8	<8
863	<8	<8	<8	<8
959	<8	<8	<8	<8
42	8	<8	<8	<8
234	16	32	64	64
411	16	<8	<8	<8
424	16	128	256	128
606	16	256	32	64
154	32	256	256	512
553	32	256	64	128
637	32	128	64	64
894	32	128	128	64
135	64	256	128	512
156	64	128	64	128
162	64	128	128	256
188	64	64	128	128
617	64	256	128	64
640	64	256	128	64
837	64	256	128	128
1222	64	256	64	256
390	128	256	128	128
410	128	256	256	512
418	128	256	128	256
1170	128	256	128	512
106	256	256	256	1024
269	256	256	256	512
333	256	128	128	64
373	256	256	256	512
449	256	256	256	512
474	256	128	128	64
1186	256	256	256	128
777	AC	256	64	128
1231	AC	<8	<8	<8

TABLE 2 - CORRELATION BETWEEN SPECIFIC ANTIBODY TITERS DETECTED IN 43 SERA BY THE CF, ELISA, IFA, AND IHA TESTS FOR CMV.

COMPARATIVE ASSAYS by:	CORRELATION COEFFICIENT (r)*
CF and ELISA	+ .41
CF and IFA	+ .54
CF and IHA	+ .56
ELISA and IFA	+ .89
ELISA and IHA	+ .90
IFA and IHA	+ .87

* Correlation coefficient (r) calculated by Spearman's rank correlation method.

FIGURE LEGENDS

Fig. 1. Comparison of CMV Antibody titers obtained in the CF, ELISA, IFA, and IHA tests.

Fig. 2. Comparison of CMV Antibody titers obtained in the ELISA, IFA, and IHA tests.

Fig. 3. Distribution and Geometric Mean Titers obtained in the CF, IFA, IHA, and ELISA tests for CMV.

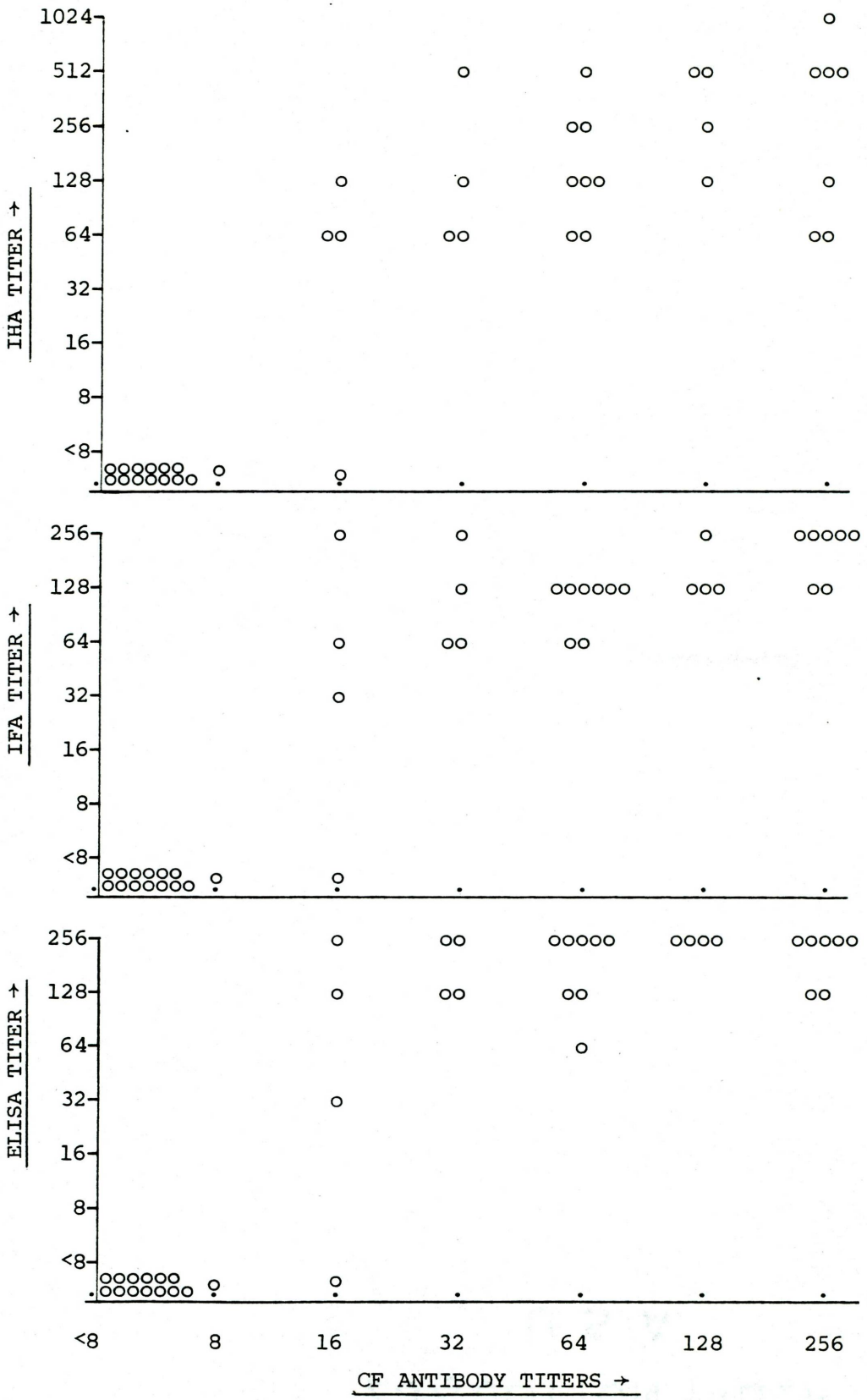
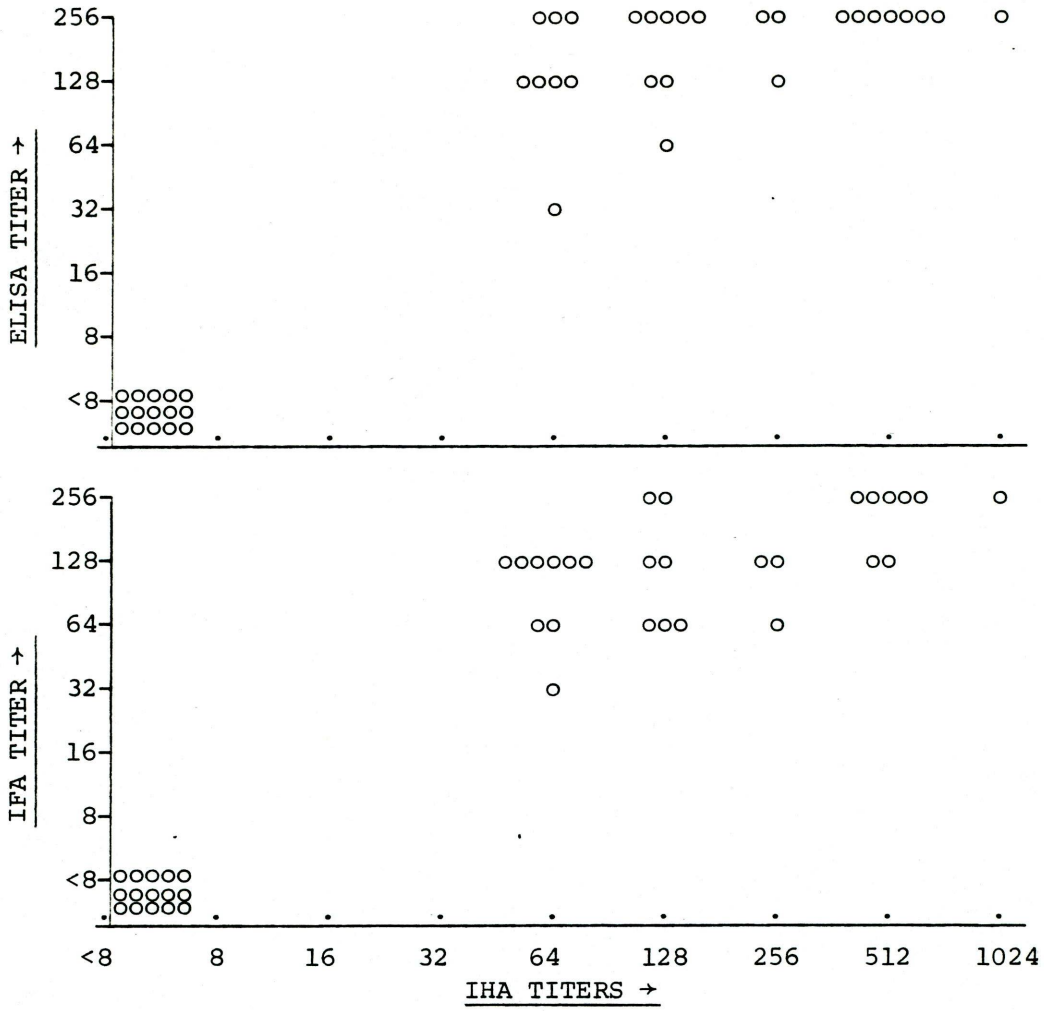

IHA TITER →
1024
512
256
128
64
32
16
8
<8
IFA TITER →
256
128
64
32
16
8
<8
ELISA TITER →
256
128
64
32
16
8
<8
<8
8
16
32
64
128
256
CF ANTIBODY TITERS →

Fig. 1


256
128
64
32
16
8
<8
ELISA TITER →
IFA TITER →
<8
8
16
32
64
128
256
512
1024
IHA TITERS →

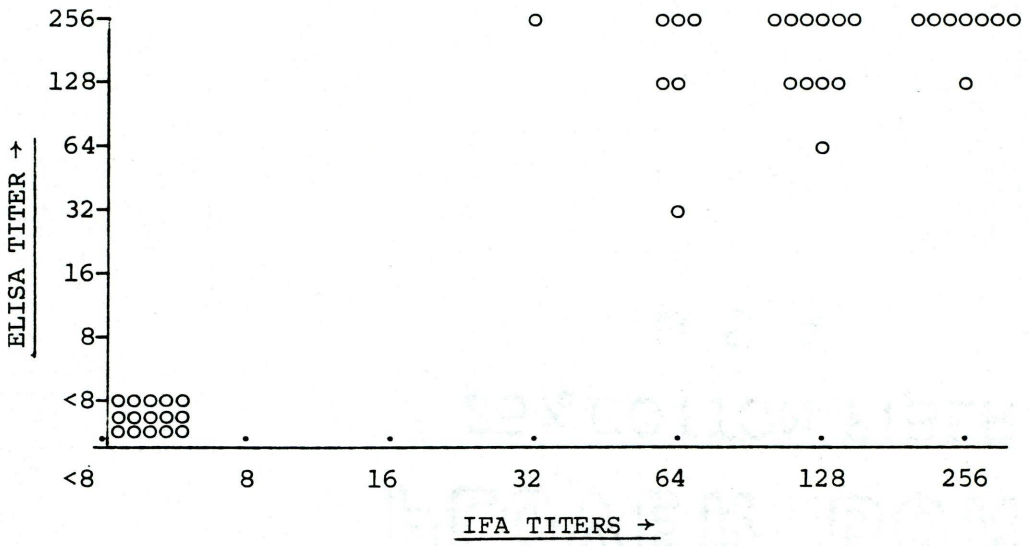

256
128
64
32
16
8
<8
ELISA TITER →
<8
8
16
32
64
128
256
IFA TITERS →

Fig. 2

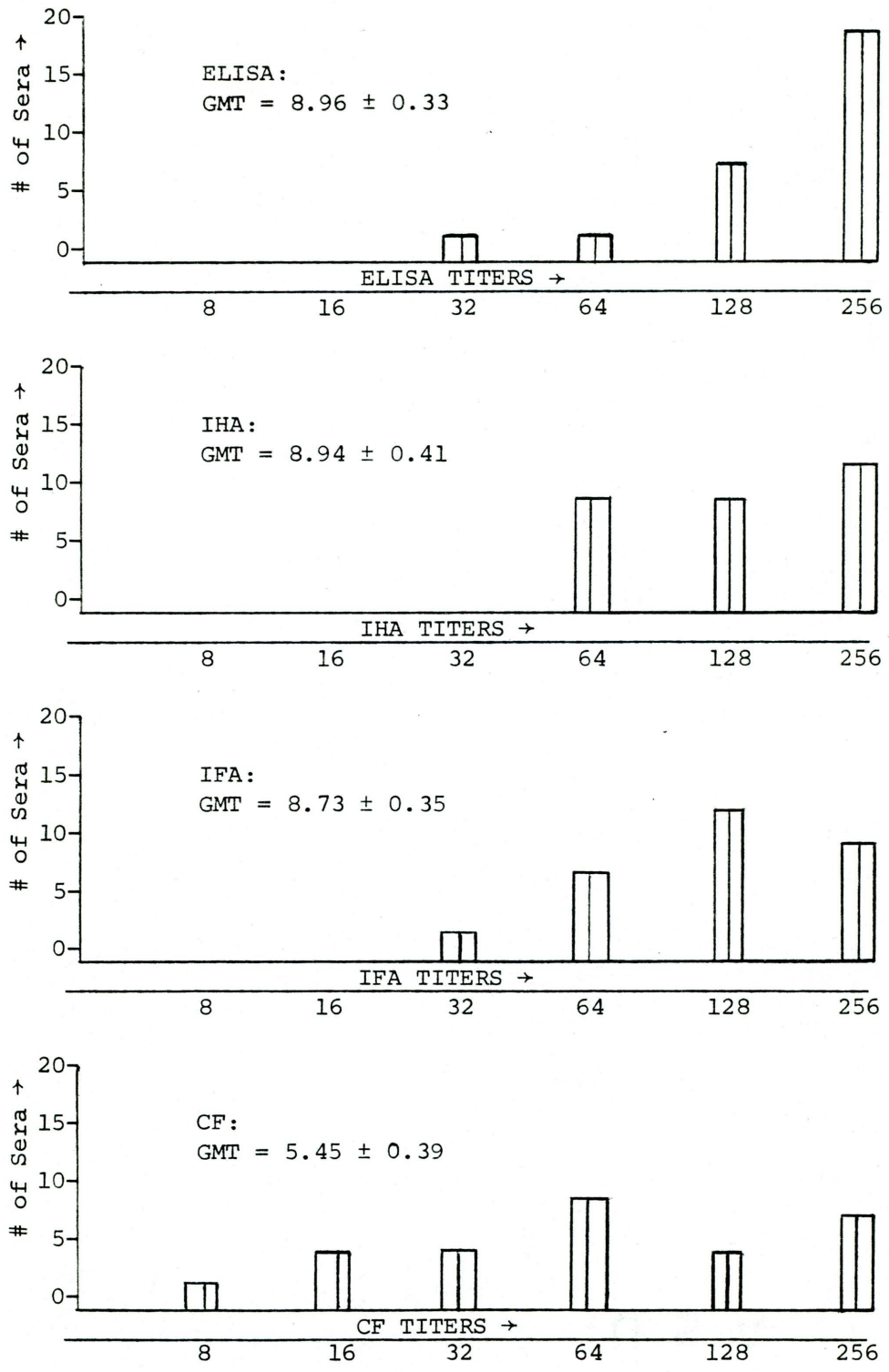


Fig. 3