JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1987, p. 1406–1410
0095-1137/87/081406-05$02.00/0

Vol. 25, No. 8

Improvement of Serological Diagnosis of Neonatal Cytomegalovirus Infection by Simultaneously Testing for Specific Immunoglobulins E and M by Antibody-Capture Enzyme-Linked Immunosorbent Assay

SØREN L. NIELSEN,[1] EBBE RØNHOLM,[1] INGER SØRENSEN,[1] PER JAEGER,[2] AND HANS K. ANDERSEN[1]*

Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus C,[1] and County Hospital, DK-7800 Skive,[2] Denmark

Received 29 December 1986/Accepted 16 April 1987

This study describes the results of testing 92 serum samples, including 10 umbilical cord serum samples, from 38 cytomegalovirus (CMV)-infected neonates for CMV-specific immunoglobulins E (IgE) and M by antibody-capture enzyme-linked immunosorbent assays with enzyme-labeled CMV antigen. All infants excreted CMV in the urine. It was demonstrated that the CMV IgE test was more sensitive than the CMV IgM test in diagnosing CMV infection in neonates by serology. Thus, the sensitivity for the IgE test was 82%, whereas for the IgM test it was only 66%. Furthermore, the CMV-specific IgE response, expressed as absorbance, was higher than the specific IgM response in 91% of the 76 sera which contained antibodies of either one or both immunoglobulin classes. Forty-six control sera, including 18 umbilical cord sera, from 46 neonates from whom CMV was not isolated were also tested. Most sera were negative. Three infants, however, had CMV antibodies of one or both classes, indicating infection. The level of total serum IgE was controlled in 24 of the sera from the CMV-infected neonates, but in none of the cases was the level elevated. No correlation was found between the reactivity of the antibodies in the two immunoglobulin classes and the level of CMV in urine.

Infections with cytomegalovirus (CMV) are relatively common among infants, although only a small proportion of infected neonates show symptoms typical of CMV disease (1, 2; K. Ahlfors, Ph.D. thesis, University of Lund, Malmö, Sweden, 1982). The infection may be either congenital or acquired, often resulting from transmission of the virus from the mother to the infant. Intrauterine transmission can occur at any time during gestation, but most infants are probably infected during birth or from the mother's milk. In developed industrial countries, the rate of congenitally CMV-infected infants is about 0.5%, whereas about 20% of infants acquire CMV infection during their first year of life (1, 2, 5, 8, 18; Ahlfors, Ph.D. thesis).

The most sensitive method of diagnosing CMV infection in neonates is isolation of virus from urine. Testing for antibodies may be hampered by the transmission of CMV antibodies of the immunoglobulin G (IgG) class from seropositive mothers to offspring. On the other hand, infected infants may themselves produce CMV antibodies of the IgM class. During the last decade several reports have appeared on the detection of CMV IgM antibodies by an immunofluorescence test, radioimmunoassay, and enzyme-linked immunosorbent assay (ELISA) (10, 17, 19). These indirect techniques, which use CMV antigen fixed to a solid phase and a fluorescein-, isotope-, or enzyme-coupled anti-IgM antibody, are of less value, because Rh factors and CMV IgG antibodies may interfere with the reactions. Therefore, sera have to be either fractionated or absorbed before use in the indirect IgM antibody tests (11). Recently, IgM antibody-capture methods for diagnosing CMV infections have been described as reliable, sensitive, and easy to perform (16, 20). They have the advantage, moreover, that preabsorption of sera is apparently not required, although nonspecific reactions may occur. Therefore, the sera must be controlled against nonviral antigens in the CMV conjugate (12).

Even with the most sensitive IgM tests, a proportion of CMV-infected infants have been found to produce either no CMV IgM antibodies at all or only very small amounts (19), making serological diagnosis difficult. However, infants also produce antibodies of the IgE class (9). This antibody class does not pass from mother to fetus and is short-lived. Recently, in a study of patients of whom most were immunocompromised transplant recipients or had hematological malignancies, van Loon et al. (21) found that specific antibodies of the IgE class occur during primary CMV infection but not during recurrent infection. We found it of interest to examine whether specific IgE antibodies are also produced in neonatal CMV infection and to compare the results with a study of the CMV IgM response by antibody-capture ELISAs with enzyme-labeled CMV antigen. As shown in this study, the CMV IgE antibody-capture ELISA is more sensitive than the IgM antibody-capture ELISA for detection of CMV antibodies in infants. In consequence, the method is now included among the tests used in our laboratory for routine diagnosis of CMV infections.

MATERIALS AND METHODS

Patients and controls. In connection with the diagnostic service and a prospective study of the incidence of congenital CMV infection (1), we collected 92 serum samples from 38 CMV-infected infants. CMV was in all cases isolated from the urine within the first 4 months of life. Serum specimens were usually available about the time that CMV excretion was detected, but in two infants with symptoms of congenital CMV infection at birth the first serum sample was taken 6 weeks before demonstration of CMV excretion. Ten of the sera were umbilical cord sera. The patients consisted of two groups: (i) 17 infants who were found to excrete CMV within the first week of life and (ii) 21 infants who were found to

* Corresponding author.

excrete CMV between the first week and the fourth month of life. The infants in the first group were infected in utero, whereas the second group probably contained both congenitally infected infants and infants with acquired CMV infection, one case of which occurred after blood transfusion.

Controls included 46 neonates less than 4 months old from whom CMV was not isolated from urine after a single attempt. The control sera were obtained within 2 weeks before or after attempted virus isolation. Eighteen of the sera were umbilical cord sera. Complement-fixing (CF) antibodies to CMV in titers from 4 to 64 were demonstrated in 31 (67%) of the 46 control sera.

Due to the ethical problems involved in obtaining blood samples from sick infants, we never requested follow-up sera after demonstration of CMV excretion, but in some cases clinicians sent additional sera. The number of serum samples studied from the 38 cases were as follows: one from 14 infants, two from 11, three from 3, four from 6, and five from 3. The interval between consecutive samples in each case was at least 14 days. From one infant eight samples, covering the period from birth to 12 months of age, were studied. All sera were inactivated at 56°C for 0.5 h and kept frozen at −20°C until use.

Diagnostic methods. Methods for isolating CMV in human lung cell (HEL) cultures and determination of CF antibodies have been published previously (1). Virus isolation was performed in glass flasks containing 50 cm^2 of HEL monolayers, which were inoculated with 0.5 ml of urine diluted with 0.5 ml of cell culture medium, and the pH was adjusted to 7.2 by dropwise addition of 2.8% sodium bicarbonate solution. The mixture was adsorbed to the cell cultures for 1 h at 37°C and afterwards exchanged with 20 ml of medium. Viruses were identified as CMV by their slowly developing cytopathic effect, resulting in production of plaques in cell culture. The number of small plaques was counted after staining with methylene blue in a colony counter (magnification, 20×) before the development of secondary plaques (ordinarily 2 weeks after the first observation of microscopic plaques), and the number of plaques was subsequently used for estimation of the virus excretion titer (PFU per milliliter of urine).

Antigens. CMV antigen was prepared from nuclei of HEL cells infected with the Ad-169 strain of human CMV essentially as reported previously (12, 20, 21). Briefly, when nearly complete cytopathic effect was observed, cells were trypsin dispersed and suspended in hypotonic buffer at 0°C for 30 min. Nonidet P-40 was added, and the cells were disrupted in a tight-fitting Dounce homogenizer. When the nuclei were largely freed from the cytoplasm, the suspension was sedimented by centrifugation, and the pellet was suspended in cold phosphate-buffered saline (PBS). The nuclei were separated from cellular debris by centrifugation (1,200 × *g* for 15 min at 4°C) through a layer of 17% Ficoll 400 in PBS. The pellet was suspended in 0.01 M sodium carbonate buffer (pH 9.5). The suspension was sonicated and clarified by centrifugation (5,000 × *g* for 10 min). The supernatant was used as the antigen for enzyme labeling. Control antigen was prepared from the nuclei of uninfected HEL cells by the same procedure.

Antigen labeling procedure. The CMV and control antigens were labeled with horseradish peroxidase by the periodate method essentially as described earlier (12, 20, 21) and used as conjugates. Only one batch of CMV conjugate and one batch of control conjugate were used in this study.

Antibody-capture ELISA. The antibody-capture ELISA for detection of CMV IgM and IgE antibodies was carried out essentially as described previously for detection of CMV IgG antibodies (12). Briefly, individual wells of ELISA plates (immunoplate I; NUNC, Copenhagen, Denmark) were coated with 0.1 ml of anti-IgM or anti-IgE (rabbit immunoglobulins to human IgM [μ chain] or IgE [ε chain]; Dakopatts, Copenhagen, Denmark) diluted 1:3,200 or 1:1,000, respectively, in carbonate buffer (pH 9.6). After 1 h of incubation at 37°C, the wells were washed in PBS containing 0.05% Tween 20, pH 7.4 (PBS-T). Then, 0.1 ml of serum diluted 1:10 in PBS-T with 2% fetal calf serum was added to each of four wells coated with either anti-IgM or anti-IgE. After incubation for 2 h at 37°C, the plates were washed in PBS-T. Two of the four wells received 50 μl of CMV conjugate per well, and the other two wells received 50 μl of control conjugate per well. The conjugates were diluted 1:1,600 for IgM and 1:2,400 for IgE in PBS-T with 2% fetal calf serum and 0.1 mg of control antigen per ml of conjugate. After incubation overnight at 4°C, the wells were washed in PBS-T. Then 50 μl of substrate solution (2 mg of *ortho*-phenylenediamine per ml of 0.1 M citrate phosphate buffer [pH 5.0] with 0.75 μl of 35% H_2O_2) was added to each of the wells. After 10 min of incubation in the dark at room temperature, the reaction was stopped by adding 0.1 ml of 1 M H_2SO_4 to each well. The A_{490} was determined by spectrophotometry (Immunoreader NJ-2000; NUNC), with A_{620} as the reference. Wells containing only 0.1 ml of H_2SO_4 were used as blanks. The specific activity was calculated as the mean absorbance of the duplicate test with the CMV conjugate.

The cutoff level was determined as the mean absorbance plus 3 standard deviations for sera from 61 CMV-negative blood donors (12) tested with the CMV conjugate. The cutoff level for CMV IgM antibodies was an absorbance of 0.2 and for CMV IgE antibodies was an absorbance of 0.1. In addition, the reactivity with the control conjugate had to be below the cutoff level for a positive reaction to be considered specific. Within-run and between-day coefficients of variation were 4 and 15%, respectively, as determined with a CMV antibody-positive serum (12).

Dilutions of sera and antigens were determined after checkerboard titrations.

Measurement of total IgE in serum. The quantitative determination of total IgE in serum was carried out by methods described previously (3, 6). We used a paper radioimmunosorbent test (Phadebas IgE PRIST; Pharmacia Diagnostics AB, Uppsala, Sweden), modified to involve one extra low standard (0.23 μg/liter). The standards were calibrated against the 1st British Standard for Human Serum IgE. The between-assay precision of the test was 5% (for concentrations between 0.23 and 46 μg/liter). The detection limit was 0.23 μg/liter.

RESULTS

CMV IgM and IgE in infants excreting CMV. The results of testing the first serum sample from each of the 38 CMV-excreting infants for CMV-specific IgM and IgE antibodies are shown from Fig. 1. Twenty-five of these sera (66%) contained CMV antibodies of both classes, and in all but two the reactivity of CMV IgE antibodies was higher than that of the CMV IgM antibodies. The difference between the reactivity of CMV antibodies in the two classes varied from case to case. In some cases it was only slight, but in nine cases IgE reactivity was more than twice as high as that of IgM. A further six sera (16%) were positive only for CMV IgE antibodies, whereas seven sera (18%) did not contain any of

the antibodies. Later sera were available for three of these negative cases, but none became positive for CMV antibodies in either the IgM or IgE class.

Of the 10 umbilical cord sera included in this material (Fig. 1), 6 were CMV IgE positive, 5 also contained CMV IgM antibodies, and 4 were completely negative.

Statistical analysis by Wilcoxon's rank sum test for the 31 sera containing one or both antibodies revealed that the CMV-specific IgE absorbance was significantly higher ($P < 0.0001$) than the CMV-specific IgM absorbance.

CMV IgM and IgE in sera from non-CMV-excreting infants. The results for the 46 control sera for CMV-specific IgM and IgE antibodies are also shown in Fig. 1. Forty-three (93%) were negative for both antibody classes. Sera from two infants, however, were slightly positive for CMV IgE antibodies only, and one infant was positive for both. Of these three infants, one was CF seronegative (titer, <4), and two had CMV CF antibodies with titers of 4 and 32. All 18 control umbilical cord serum samples were negative.

CMV IgM and IgE in later samples from infants excreting CMV. The remaining material, from infants up to 24 months of age, was also tested for CMV-specific IgM and IgE, and in this material too, the CMV IgE test was the most sensitive. The results for 92 serum samples covering the period from 0 to 24 months of age are given in Table 1. Of the 92 sera, 76 contained either CMV IgM, CMV IgE, or both. Sixteen sera (17%) were positive only for CMV IgE, and one serum was positive only for CMV IgM. In 69 (91%) of the 76 sera, the reactivity of CMV IgE was higher than the reactivity of CMV IgM antibodies, and this phenomenon was independent of age (Table 1). The number of sera which were negative for CMV antibodies of both classes increased with age, as 43% of the sera from infants more than 6 months of age were negative for antibodies of either class.

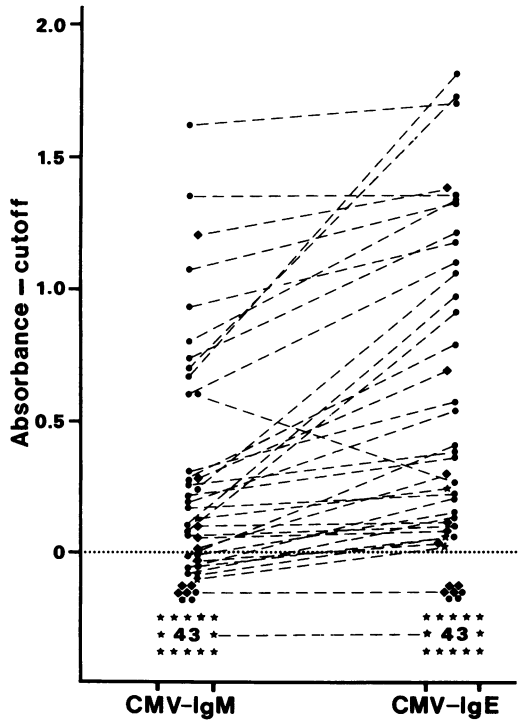

FIG. 1. CMV IgM and IgE absorbance of serum samples from 38 CMV-infected infants less than 4 months of age and 46 controls. Symbols: ◆, umbilical cord sera from infected infants; ●, sera from infected infants less than 4 months of age; ★, control sera, including 18 umbilical cord sera.

TABLE 1. Detection of CMV IgE and IgM in serum samples from 38 CMV-excreting infants by antibody-capture ELISA

Age (mo)	No. of samples	No. of samples (%)		
		Negative for IgE and IgM	Positive for IgE, IgM, or both	With IgE > IgM[a]
0–1	26	6 (23)	20	19 (95)
1–3	29	3 (10)	26	23 (88)
3–6	21	0 (0)	21	19 (90)
6–24	16	7 (43)	9	8 (89)
0–24	92	16 (17)	76	69 (91)

[a] Absorbance minus cutoff.

Total IgE in infants excreting CMV. Twenty-four serum samples from seven of the CMV-excreting infants were tested for total IgE (data not shown). Sera were taken from 1 day to 6 month of age. Two of the infants had only a very slight CMV IgE antibody response, whereas the remaining five infants were clearly positive for CMV IgE. The level of total IgE in the 24 sera was between <0.23 and 18.4 μg/liter. According to the standard for this age group, none of the infants had elevated levels of total IgE (4, 9).

Relationship between CMV IgM and IgE antibody reactivity and level of virus excretion. The results of analyzing whether there was any correlation between the presence and reactivity of the two antibody classes and the antigen burden, as determined by CMV excretion level, appear in Fig. 2. No correlation was found by comparing either the initial specimens from the infants (Fig. 2) or the antibody reactivity of the two classes in later blood specimens with later virus excretion levels (data not shown).

DISCUSSION

This study demonstrates the results of testing sera from 38 CMV-infected neonates for both IgE and IgM antibodies to CMV by antibody-capture ELISAs.

Surprisingly, the reactivity of specific IgE antibodies, expressed as absorbance, was found to be higher than the specific IgM response in about 90% of the positive sera from CMV-infected infants (Table 1). This was especially unexpected because the total amounts of these immunoglobulin classes are so different, the level of IgM during the first 6 months of life being on the order of 1.1 to 4.3 g/liter and the level of IgE being between ≤2.3 and 25 μg/liter (9).

The advantage of including a test for CMV antibodies of the IgE class in the diagnosis of CMV infection in neonates

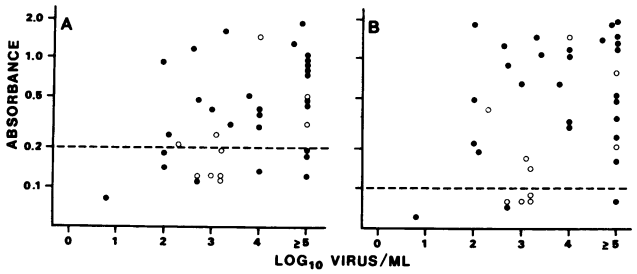

FIG. 2. CMV IgM (A) and IgE (B) absorbance in initial serum samples from 38 CMV-infected infants with different virus excretion levels. Symbols: ○, umbilical cord sera; ●, sera from infants less than 4 months of age. Dotted lines indicate cutoff levels.

is best illustrated in the study of the initial samples (Fig. 1). Of the 38 sera from infants excreting CMV, 25 were positive in both tests, whereas 6 sera contained CMV IgE antibodies only. Thus, by using the IgE test, a serological diagnosis was possible for 82%, whereas with the IgM test alone a serological diagnosis of the infection would only have been possible for 66%.

It would have been more simple just to replace the test for IgM antibodies with the test for IgE antibodies, but as seen in Fig. 1, this is not advisable, as one sample had higher reactivity in the IgM than in the IgE test. Moreover, one of the late sera was IgM positive but IgE negative.

With increasing age there is a tendency in these early-infected infants to a decrease in both classes of antibodies, as 43% of the sera taken after the age of 6 months were negative in both tests (Table 1).

None of the sera tested reacted against the control conjugate, indicating that nonspecific reactions are not a problem in the serological diagnosis of neonatal CMV infections by antibody-capture ELISA. Reactivity against control conjugates is, however, sometimes seen with sera from both CMV-infected patients (children and adults) and patients without CMV infection. We have observed nonspecific reactions with control conjugates more frequently in the CMV IgM antibody-capture ELISA than in the IgE test, which further indicates that it is useful to include tests for CMV IgE antibodies in the routine diagnosis of CMV infections.

It is generally accepted (10) that the most sensitive diagnostic test for congenitally and neonatally acquired CMV infection is isolation of CMV from urine. This, however, is a time-consuming and cumbersome test demanding cell cultures. Furthermore, isolation of CMV is not a 100% efficient test, as CMV may only be intermittently present in the urine of infected infants (7). In this respect, it is interesting that three (7%) of our control infants who were found not to be excreting CMV showed positive reactions in one or both tests (Fig. 1). This was probably not because of nonspecific reactions, as no reaction was observed with the control conjugate. It was probably also not a question of persistence of maternal IgG antibodies, as only two of them were CMV CF positive, with titers of 4 and 32, titers which were also found among 67% of the CMV IgE- and IgM-negative control infants. These infants were most likely infected, although CMV by chance was not detected in the urine.

Why is the specific reactivity higher in the IgE test than in the IgM test? First, the cutoff level in the IgM test is twice as high as that in the IgE test because the reactivity of control sera with CMV conjugate is higher in the IgM test. We have tried to reduce the background by different methods, but it has not been possible to get below 0.2 for the cutoff level of the IgM test, a level which is comparable to that found by others (15, 16, 19, 20). Thus, some of the increased sensitivity in the IgE test is attributable to the difference in the backgrounds in the two tests. Second, it seems in some cases to be a question of relatively higher amounts of specific IgE than of IgM antibodies, as the reactivity of nine sera was considerably higher in the IgE than in the IgM test. We have no explanation for these individual variations, but they do not seem to depend on the antigenic burden, as evaluated by virus excretion levels (Fig. 2). Neither does it seem to be a question of stability of the two classes of antibodies at −20°C, as specific antibodies of both antibody classes were detectable in sera after storage for up to 10 years.

Certain viral infections are known to produce specific IgE antibody, to the extent that significant changes in the level of total serum IgE may occur (3, 13–15, 22). However, although we controlled the level of total IgE in serum for some of our patients when sufficient amounts of serum were available, the level did not in any case exceed the levels normally found in infants of the same age (4, 9).

The conclusion of this study is that detection of CMV IgE and IgM antibodies by antibody-capture ELISA is a sensitive and specific means of diagnosing CMV infection in neonates. Detection of CMV IgE in particular is of great value. The specific CMV IgE response can probably also be demonstrated by other sensitive techniques (ELISA, radioimmunoassay). In addition, detection of specific IgE probably will be of great value in the diagnosis of other congenital infectious diseases (15). We have not yet investigated these possibilities.

LITERATURE CITED

1. **Andersen, H. K., K. Brostrøm, K. B. Hansen, J. Leerhøy, M. Pedersen, O. Østerballe, U. Felsager, and S. Mogensen.** 1979. A prospective study on the incidence and significance of congenital cytomegalovirus infection. Acta Paediatr. Scand. **68**:329–336.
2. **Andersen, H. K., J. J. Gravesen, and T. Iversen.** 1972. Cytomegalovirus infection among infants admitted to a paediatric department. Acta Paediatr. Scand. **61**:445–451.
3. **Bahna, S. L., C. A. Horwitz, M. Fiala, and D. C. Heiner.** 1978. IgE response in heterophil-positive infectious mononucleosis. J. Allergy Clin. Immunol. **62**:167–173.
4. **Bhalla, R. B., I. Rappaport, I. Defilippi, and M. K. Schwartz.** 1982. Serum IgE levels in a northeast United States caucasian population, p. 295–305. *In* A. A. Bensen (ed.), Advanced interpretation of clinical laboratory data. Marcel Dekker Inc., New York.
5. **Carlstöm, G.** 1965. Virologic studies on cytomegalic inclusion disease. Acta Paediatr. Scand. **54**:17–23.
6. **Ceska, M., and U. Lundkvist.** 1972. A new and simple radioimmunoassay method for the determination of IgE. Immunochemistry **9**:1021–1030.
7. **Diosi, P., L. Babusceac, O. Nevinglovschi, and A. Stoicanescu.** 1966. Duration of cytomegaloviruria in nursery children. Pathol. Microbiol. **29**:513–518.
8. **Friis, H., and H. K. Andersen.** 1982. Rate of inactivation of cytomegalovirus in raw banked milk during storage at −20°C and pasteurisation. Br. Med. J. **285**:1604–1605.
9. **Fudenberg, H. H., D. P. Stites, J. L. Caldwell, and J. V. Wells.** 1976. Basic and clinical immunology, p. 210, 285. Lange, Los Altos, Calif.
10. **Griffiths, P. D., S. Stagno, R. F. Pass, R. J. Smith, and C. A. Alford.** 1982. Congenital cytomegalovirus infection: diagnostic and prognostic significance of the detection of specific immunoglobulin M antibodies in cord serum. Pediatrics **69**:544–549.
11. **Joassin, L., and M. Reginster.** 1986. Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin G. J. Clin. Microbiol. **23**:576–581.
12. **Nielsen, S. L., E. Rønholm, I. Sørensen, and H. K. Andersen.** 1986. Detection of immunoglobulin G antibodies to cytomegalovirus antigens by antibody capture enzyme-linked immunosorbent assay. J. Clin. Microbiol. **24**:998–1003.
13. **Nordbring, F., S. G. O. Johansson, and Å. Espmark.** 1972. Raised serum levels of IgE in infectious mononucleosis. Scand. J. Infect. Dis. **4**:119–124.
14. **Perelmutter, L., L. Potvin, and P. Phipps.** 1979. Immunoglobulin E response during viral infections. J. Allergy Clin. Immunol. **64**:127–130.
15. **Salonen, E.-M., T. Hovi, O. Meurman, T. Vesikari, and A. Vaheri.** 1985. Kinetics of specific IgA, IgD, IgE, IgG, and IgM antibody responses in rubella. J. Med. Virol. **16**:1–9.
16. **Schmitz, H., U. von Deimling, and B. Flehmig.** 1980. Detection of IgM antibodies to cytomegalovirus (CMV) using an enzyme-

labelled antigen (ELA). J. Gen. Virol. **50**:59–68.

17. **Stagno, S., R. F. Pass, D. W. Reynolds, M. A. Moore, A. J. Nahmias, and C. A. Alford.** 1980. Comparative study of diagnostic procedures for congenital cytomegalovirus infection. Pediatrics **65**:251–257.
18. **Stagno, S., D. W. Reynolds, R. F. Pass, and C. A. Alford.** 1980. Breast milk and the risk of cytomegalovirus infection. N. Engl. J. Med. **302**:1073–1076.
19. **Stagno, S., M. K. Tinker, C. Elrod, D. A. Fuccillo, G. Cloud, and A. J. O'Beirne.** 1985. Immunoglobulin M antibodies detected by enzyme-linked immunosorbent assay and radioimmunoassay in the diagnosis of cytomegalovirus infections in pregnant women and newborn infants. J. Clin. Microbiol. **21**:930–935.
20. **van Loon, A. M., F. W. A. Heessen, J. T. M. van der Logt, and J. van der Veen.** 1981. Direct enzyme-linked immunosorbent assay that uses peroxidase-labeled antigen for determination of immunoglobulin M antibody to cytomegalovirus. J. Clin. Microbiol. **13**:416–422.
21. **van Loon, A. M., J. T. M. van der Logt, F. W. A. Heessen, and J. van der Veen.** 1985. Quantitation of immunoglobulin E antibody to cytomegalovirus by antibody capture enzyme-linked immunosorbent assay. J. Clin. Microbiol. **21**:558–561.
22. **Welliver, R. C., D. T. Wong, E. Middleton, M. Sun, N. McCarthy, and P. L. Ogra.** 1982. Role of parainfluenza virus specific IgE in pathogenesis of croup and wheezing subsequent to infection. J. Pediatr. **101**:889–896.