RAPID DETECTION OF IgM AND IgG ANTIOBODIES TO HERPESVIRIDAE VIRUS

BY COLORZYME EA TEST IN COMPARISON WITH CFT, IF AND ELISA TESTS

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ABSTRACT

In this study, the Immunoconcepts EA indirect enzyme antibody technique (colorzyme) was used not only for detection of IgG antibodies but also for quantitative detection of IgM antibodies to Herpes Simplex Virus (HSV), Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) to diagnose recent ìactiveî infection.

Reference reactive and negative antisera and randomly collected human sera were tested by complement fixation test (CFT) against HSV antigens and tested also by immunofluorescent (IF) and colorzyme Immunoconcepts EA tests.

All sera that were negative to HSV, CMV and EBV antibodies by CFT were negative by IF and colorzyme EA tests. All antibody positive sera and reference positive antisera were also positive by IF and colorzyme EA tests with slight variation in antibody titres between CFT and colorzyme test results.

Human sera which were negative or IgM positive to HSV, CMV and EBV by ELISA as well as negative and positive reference sera from different diagnostic kits were retested by IF and colorzyme EA for IgM antiviral reactivity results were concordance byt the three rests.

All incubations in colorzyme test were at room temperature and only an ordinary microscope used in IF test or plate washers and readers needed for ELISA test.

The colorzyme immunoconcepts is a simple, rapid and sensitive for viral diagnosis and can be used in any private laboratory.

INTRODUCTION :

Herpes Simplex Virus (H. hominis) type 1 and 2 (HSV 1 and 2) are responsible for some of the most common wide range of clinical conditions in man ranging from mild localized lesions to severe fatal disseminated diseases and encephalitis (Nahmias and Dowdle, 1968). The most common sites of infection are the oral activity, eyes, skin, fingers and genitalia, IgM antibody is the first antibody to develop in response to primary HSV infection and is followed by the production of IgG antibody (Rasmussen et al. 1982). However, HSV tends to cause persistent latent infection with frequent episodes of reactivation with the development of acute lesions.

The presence of HSV, IgM antibody indicates recent either primary or recurrent HSV infection. In secondary infection (reactivation of latent virus or reinfection) not all patients show an IgM response but usually in severe reaction, IgM antibodies are produced again as in primary infection. The total antibody in testing paired samples should be determined additionally and fourfold increase in IgG titer may indicate active infection. Early detection of IgM antibody is important in patient management and treatment (Marettila and Kalimo, 1977 and Gallo et al., 1981).

Cytomegalovirus (CMV) which is one of the herpes virus family is widely distributed in human. Infection by CMV is generally mild or sub clinical (Adler, 1986) but fatal cytomegalic inclusion disease can be observed in congenitally infected newborn in immune suppressed patients.

The presence of serum IgMantibodies to CMV in newborns is suggestive of congenital infection and an increase in the titre of IgM antibody to CMV during the first year of life also can be diagnostic (Grffiths et al., 1982 and Stagno et al., 1985).

CMV IgM can be used as an aid to diagnose recent primary or recurrent CM infection.

EBV an antigenically distinct human herpes virus was originally observed in cell cultures of Burkitte lymphoma (B.L.). (Epestein and Achong, 1973). The virus has been also found in many lymphoid cell cultures derived from patients with BL, nasopharyngeal carcinoma, infectious mononucleosis (IM) as well as from normal individuals (Henle and Henle, 1972).

EBV infected lymphoblastoid cells in culture contain EBV early Ag (EA), EBV nuclear Ag (EBVNA), virus capsid Ag (VCA) and a virus speccified membrane Ag (MA) (Henle et al. 1971). These antiges are measured by immunofluoresence (IF) and complement fixation (CF) tests using hyper immune serum.

In infectious monocleosis, both IgG and IgM antibodies to EB-VCA develop rapidly. The IgM antibody disappears within 8 to 10 weeks.

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(Nickoskelainen and Hanninen, 1975), whereas IgG antibody declines to lower titers which persist indefinitely (Tischenorf et al., 1970). Specific IgM antibody to EB-VCA appears within 1 to 2 weeks after onset of clinical symptoms and drops to undetectable levels within 8 to 10 weeks. The EB-VCA IgM assay is particularly helpful when only one srum specimen is available for determine the presence of current recent infection.

The aim of this study is to compare the results of colorzyme EA (a new immunoconcepts diagnostic test for IgM and IgG antibody detection), test for IgM antibody presence in recent infection with those assays include indirect IF and enzyme immunoassay (ELISA) beside comparing the results of colorzyme test for IgG detection to HSV, with those of CF and IF tests.

MATERIAL AND METHODS

1 Human sera tested

For HSV IgG antibody detection, 38 human sera tested by complement fixation (CF0, immunofluorescent (IF) and colorzyme tests beside 6 positive reference sera from commercial kits. For IgM antibodies to HSV, 34 human sera and 6 reference positive sera were tested by ELISA, IF and colorzyme tests.

For CMV IgG antibody detection, 55 human sera and 9 negative and 10 positive reference sera from different commercial kits (Abbott, Guull, Virion, Organon, Kallestad, Serion) were tested by ELISA, IF and colorzyme EA tests.

For EBV IgG antibody detection, 26 human sera and one negative and one positive reference sera were tested by CF, IF and Colorzyme EA tests while for EBV IgM antibody detection, 26 human sera were tested by ELISA, IF, and Colorzyme, EA tests.

2 Techniques used

Complement Fixation Test

The micro CFT was used (Edwin and Nathalie, 1979). The sera were tested in a 1/8 dilution.

Immuno fluorescent (IF)

Indirect fluorescent antibody method was used for detection of IgM and IgG antibodies to HSV, CMV and EBV, viruses acoording to the instructions of manufacturers. Fixed antigen substrate slides were (Gull, Immuno-concepts, Kallestad, USA). Reference negative and positive control sera for IgG and IgM were from the commercial kits produced by Abbott, Gull, and Viron. The test was done according to the procedures described by Shley 1993, Gallo 1981 and Marettila and Kalima 1977.

For CMV, IgM ddetection (Langenhugsen et al, 1970) techniques were used. For EBV IgG antibody detection (Nikoskelainen and Hannunon, 1975). Anti-human IgG and IgM Fluorescent labelled conjugates (Pasteur Laboratories), were used.

Enzyme linked immunsorbant assay (ELISA)

The Abbott, USA, CMV-M EIA test kit was used. It is a solid phase enzyme immuno assay for the detection of IgM antibody to CMV in human sera (Grifiths et al, 1982).

For HSV - IgM detection, Serion, ELISA classic kit was used which is an indirect ELISA for the detection of specific IgM antibodies to HSV in human sera.

Colorzyme EA Test

The immuno concepts EBV-EA test used the indirect enzyme antibody technique. Diluted patient serum samples were incubated with EBV infected cells fixed on to glass microscope slides, to allow specific binding of anti-EBV-EA antibodies. If EBV-EA antibodies are present, the antibodies bind to viral early antigen, which present in 5-10% of the cells. After washing to remove non-specifically bound antibodies, the substrate was incubated with anti-human IgG conjugated to horseradish peroxidase (HRP). When the results are positive there is a formation of stable threepart complex consisting of HRP-conjugated anti-human antibody bound to human anti-EBV-EA IgG antibody which is bound to EBV early antigen located in the infected cells. This complex is visualized by incubating the slide in reagent which contains an enzyme specific substrate, the reaction between the enzyme labelled antibody and enzyme ustrate results in a color reaction visible by standard light microscopy examination of the slide. In positive samples, 10% of the cells will demonstrate dark blue-purple staining of the entire cell or cytoplsm. If the sample is negative for EBV-

Table 1
Detection of IgG antibodies to HSV, in patient sera using complement fixation, Immunofluorescent and colorzyme EA tests

Virus	No of sera Tested	Specific IgG detection					
		CF		IF		Colorzyme	
		-ve	+ve	-ve	+ve	-ve	+ve
HSV[1]	38	9	29	9	29	9	29
Reference [2] +ve HSV sera	6	-	6	-	6	-	6
CMV [1]	30	3	27	3	27	3	27
Reference [2] + ve CMV serum	1	-	1	-	1	-	1
1 EBV [1]	26	10	16	10	16	10	16
Reference [2]							
Negative	1	1	-	1	-	1	-
Positive anti EBV serum 26	1	-	1	-	1	-	1

1. Patient tested sera
2. Reference positive and negative sera from different commercial proceducers

Table 2
Detection of IgM antibodies to HSV in patient sera in case of recent active infection using ELISA, IF and Colorzyme tests

Virus	No of sera Tested	Specific IgG detection					
		ELISA		IF		Colorzyme	
		-ve	+ve	-ve	+ve	-ve	+ve
HSV[1]	34	9	25	9	25	9	25
Reference [2] Positive sera	3	-	3	-	3	-	3
CMV [1]	55	18	37	18	37	18	37
Reference[2]							
Negative	9	9	-	9	-	9	-
Positive anti-CMV	10	-	10	-	10	-	10
EBV1	26	24	2	24	2	24	2

(1) Patient sera
(2) Reference negative and positive sera from different commercial producers (Abbott, Gull, Virion, Organon, Kallestad, Serion).

EA, specific staining of the antigen-containing cells will not be seen. Since only 5-10% of the cells contain early antigen, the remaining 90-95% of the cells can be used as a check for non-specific staining.

CMV infected cells slides were used for detection of IgM and IgM to HSV (Immuno-concepts, USA).

CMV infected cells slides were used for detection of IgM and IgM to CMV (Gull, USA). HSV infected cell slides were used for detection of IgM and IgG to HSV (Immuno-concepts, USA).

For detection of IgM antibodies, anti-human IgG conjugated with horseradish peroxidase was used. The enzyme substrate and its diluent (Abott, USA) were used.

Results

HSV, IgG detection :

Out of 38 human sera tested by CFT for detection of HSV antibody, 29 were positive beside 6 reference positive sera from different laboratories.

Testing these sera by IF and colorzyme EA, the same results were obtained and all negative HSV human sera negative by IF and colorzyme tests and all positive human sera and reference sera positive by IF and Colorzyme and only slight variation in antibody titre were observed between CFT and colorzyme tests. (table 1) (Figure 1 A,B).

HSV, IgM detection :

34 human sera were tested by ELISA of which 25 sera were positive. These sera beside 3 references positive (ELISA Kits) were tested by IF and colorzyme tests and the results by the 3 techniques were identical. (Table 2) and (Figure-1 c,d).

CMV IgG Detection :

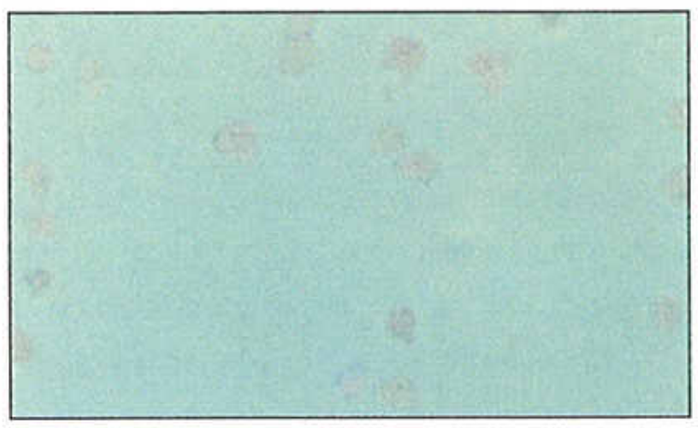
Out of 30 human sera tested, 27 were positive by CF test when these sera were tested by IF and colorzyme tests beside reference positive anti sera, the results were the same in the 3 techniques (table 1).

CMV IgM Detection :

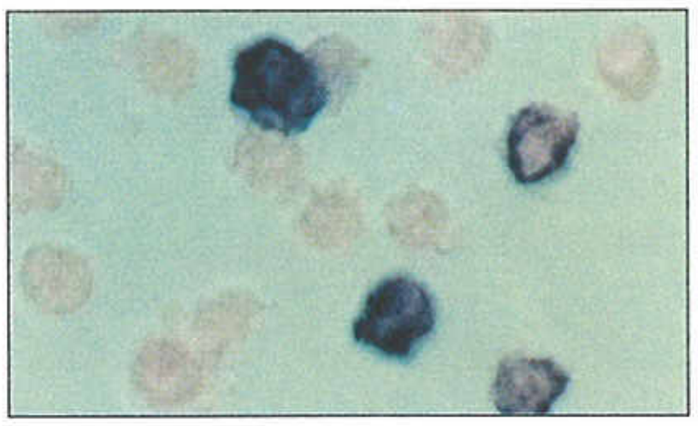
When 55 sera were tested primarly by ELISA, 37 gave positive results for CMV IgM.

All these sera (negative and positive ELISA) beside 9 negative and 10 positive reference sera from different kits were tested by IF and colorzyme for IgM detection and the results were the same obtained by ELISA test (Table 2).

A : Human sera negative by CF test to EBV was negative by colorzyme test (x20). All EBV infected cells (10%) and noninfected cells (90%) were negative (faint staining).

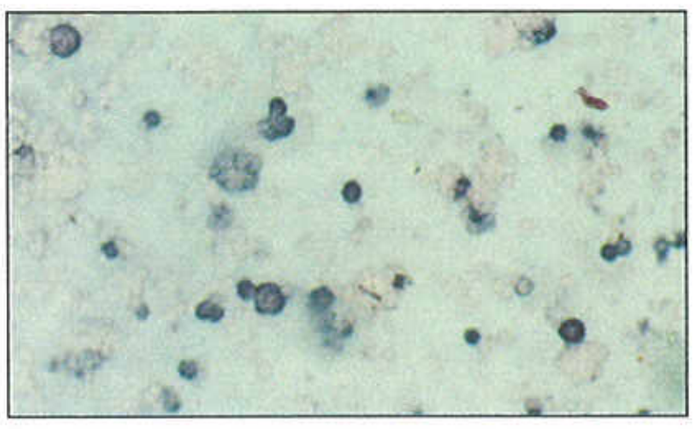


B : Human sera positive by CF test to EBV was positive by colorzyme test (x40). EBV infected cell (10%0 showed dark blue-purple staining.



C : Human sera negative by ELISA to HSV. IgM was negative by colorzyme IgM test (x10).

D :Human sera positive by ELISA IgM to HSV was positive by colorzyme IgM test (x10). HSV infected cells (about 10%) showed dark blue, purple staining.



EBV IgG Detection :

Out of 26 hyman sera tested by CF test, 16 were positive for EBV. All theses sera beside one negative and one positive. Reference sera weretested by IF and colorzyme test and the results were the same in the techniques (Table 1) and (Figure 1 - a,b).

EBV IgM Detection :

26 human sera were tested and 2 of which were positive ELISA. When these sera were tested by IF and colorzyme tests, these 2 positive sera alone were also positive by these 2 other techniques (Table 2).

DISCUSSION

For the development of reliable test for quantitative detection of IgG and IgM antibodies to HSV rapidity and specificity of the test is very important besides it must be economic.

Till now, such a test has not been routinely available in developing countries, in which no fluorescent microscopes, incubators, plate washers and spectro-photometric readers for ELISA beside other expensive equipments are not available.

For the other tests used, IF and ELISA are two accurate tests used for detection of IgG and IgM antibodies to HSV, EBV, CMV and many other viruses. CF test can be of limited diagnostic value unless testing paired sera. If neonatal infection is suspected, HSV IgM test of a single serum sample can be very useful in establishing specific etiology. IgM antibodies can not be transmitted through the placental membrane from the mother, therefore any HSV, IgM antibody present in the neonate has been produced by the infant in response to a current active infection. (Nahmias and Visntine, 1976).

Our results using colorzyme EA - M test for detection of IgM antibodies to HSV was completly identical with those IgM ELISA and IF test and all negative human sera by ELISA were negative by IF and colorzyme EA tests and all positive human sera by ELISA to HSV were also positive by IF and colorzyme EA tests. Many negative and positive reference antisera from different diagnostic kits when tested by the 3 techniques gave concordant results.

Although, serological response to HSV infection has been intensively studied (Jordan and Rytel, 1981, Kuhn et al, 1986), yet several aspects remain unclear like the immune response of patients with recurrent infection. The use of recently introduced western blot beside ELISA for testing primary and recurrent HSV type 1, infection (Rabie-Finger et al, 1991), aimed at the differential diagnosis of both courses of HSV infection.

IgM detection in primary or recurrent infection is very important, itís presence is the best indicator of active infection. Detection of IgM antibodies in patients with recurrent HSV infection has been previously reported in the last 20 years (Juto and settergen, 1988, Rabie-finger et al, 1991), they suggested that the presence of HSV IgM may be related to the severity of thi infection.

Also, IgM antibodies has been detected in patients with recurrent infections by other herpes virus such as CMV (Van Loon et al, 1985).

The presence of serumIgM antibodies to CMV in newborns is suggestive of congenital infection which can be life thretening with surviving infants often left with permanent neurologic manifestations.

CMV infection cau rarely be diagnosed with certainly on clinical grounds alone but conclusive evidence of active infection can be obtained by detection of IgM antibodies.

An increase of the titre of IgM antibodies against CMV during the first year of life can be diagnosed (Stangno et al, 1985).

Our results of colorzyme EA test were identical with IF and ELISA when negative and positive human sera to CMV tested by ELISA were tested by IF and colorzyme EA tests. Also reference negative and positive IgM to CMV of different diagnostic kits were tested by the 3 techniques and the results were the same.

The same results were obtaine when testing human sera for EBV-IgM detection.

Since specific IgM antibody to EBV-VCA appears within 1 to 2 weeks after the onset of clinical symptoms and drops to undetectable levels within 8-10 weeks, the EB-VCA IgM class assay is particularly helpful when only one serum specime is available for determining the presence of active current infection (Nickoskelainen and Hanninen, 1975).

Serology is the only way to identify EBV infection in the absence of typical diagnostic symptoms of infectious mononucleosis. EBV serology is important in diagnosing infection in young children since they usually do not develop typical symptoms or heterophil antibodies.

Our results showed that sensitivity and specificity of colorzyme EA test was found to be the same like IF and CF test for detection of IgG antibodies against HSV, and also the same like ELISA and IF for detection og IgM antibodies to that virus in recent active infection.

In colorzyme test, all incubations are done at room temperature, and only an ordinary microscope which is expected to be available in any private laboratory in developine countries, can be used where most of the expensive equipments are not available.

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