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PRELIMINARY EVALUATION OF CHORUS SYSTEM IN COMPARISON WITH MINI-VIDAS SYSTEM FOR DETECTION OF CYTOMEGALOVIRUS-IgM ANTIBODIES

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ABSTRACT

Cytomegalovirus is a herpes virus transmitted by close human contact. No symptoms of infection are apparent in majority of cases. However, the virus is very dangerous and is spread via fluids of the body especially to the new born baby from an infected mother. Medical diagnostic is working to determine the most sensitive techniques for the detection of Cytomegalovirus antibodies, in the framework of which is developed this scientific work. An enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument) and an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) have been compared with each other for the detection of Cytomegalovirus IgM antibodies. There have been analyzed 198 samples with each technique. 190 out of 198 samples (96%), gave compatible results. In particular, 153 samples gave negative results, 35 samples gave positive results and 2 samples gave doubtful results with both techniques. It was observed that 3 samples were positive in Vidas instrument and negative in Chorus instrument and 5 samples were positive in Vidas instrument and doubtful in Chorus instrument. Comparative evaluation of the two assays demonstrated a comparable sensitivity for all systems. ELFA technique showed a better ability to detect Cytomegalovirus IgM antibodies during the early stage of acute infection. Analysis of the results revealed a good level of concordance between the two assays and confirmed the usefulness of ELFA technique to diagnose acute cytomegalovirus infection.

KEYWORDS: ELISA, ELFA, Cytomegalovirus IgM

INTRODUCTION

Cytomegalovirus, a member of the herpes virus family, is ubiquitous in all human populations, causing infections which are followed by life-long latency in the host with occasional reactivations as well as recurrent infections. The seroprevalence of antibodies in adults ranges from 40-100% with inverse correlation to socioeconomic status **(Genser, at. al)**. Transmission of infection requires intimate contact with infected excretions such as saliva, urine, cervical and vaginal excretions, semen, breast milk and blood. CMV infections are usually mild and asymptomatic. However, primary maternal CMV infection during pregnancy carries a high risk of intrauterine transmission which may result in severe fetal damage, including growth and mental retardation, jaundice and CNS abnormalities **(Revello, at. al)**. Those who are asymptomatic at birth may develop hearing defects or learning disabilities later in life. A first step in diagnosing acute primary CMV infection is most commonly made by the detection of anti-CMV-specific IgG and IgM antibodies. Samples being reactive for IgM antibodies indicate an acute, recent or reactivated infection **(Genser, at. al)**. Medical diagnostic is working to determine the most sensitive techniques for the detection of *Cytomegalovirus* antibodies, in the framework of which is developed this scientific work. In this study is preliminary evaluated the Chorus system in comparison with Vidas system for the detection of Cytomegalovirus IgM antibodies. The assay of specific IgM is of great importance in the diagnosis of primary infection.

MATERIAL AND METHODS

An enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument) and an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) have been compared with each other for the detection of Toxoplasma IgM antibodies. There have been analyzed 200 patients with each technique. The serums of patients were collected using tubes containing separating gel in the normal manner from the vein.

Principle of ELISA Technique

This test is applied on CHORUS instrument, which is a new device in medical diagnostics. The test is based on the ELISA principle. The partially purified Cytomegalovirus antigen is bound to the solid phase. Through incubation with human serum diluted in a diluent which blocks the IgG, the specific IgM are bound to the antigen. After washing to eliminate the proteins which have not reacted, the sample is incubated with the conjugate composed of monoclonal anti-human IgM antibodies labelled with peroxidise. The unbound conjugate is eliminated and the peroxidase substrate is added. The colour which develops is proportional to the concentration of specific antibodies present in the serum. The disposable devices contain all the reagents to perform the test when applied on the CHORUS instrument. The control serum is used to check the validity of the results obtained. It should be used as reported in the operating manual. If the instrument signals that the control serum has a value outside the acceptable range, the calibration must be repeated. The previous result will be automatically corrected.

Description of Cytomegalovirus Strip

The strip consist of 7 wells covered with a labelled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the undiluted sample. The wells in the center section of the strip contain the various reagents required for the assay.

Specimen Type and Collection

Human serum collected in separating tube gel in the normal manner from the vein and handled with all precautions. Samples can be stored at 2-8°C for 4 days, or frozen for longer periods at -20°C.

Principle of ELFA Technique

This technique is applied in MINI-VIDAS instrument. The assay principle combines a two step enzyme immunoassay sandwich method with a final fluorescent detection. The solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready to use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. Anti CMV IgM antibodies present in the serum will bind to the CMV antigen coating the anterior of the SPR. Unbound components are eliminated during the washing steps. An Alkaline phosphatase-labeled monoclonal anti-human IgM antibody is cycled in and out of the SPR. A final wash step removes unbound components. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450nm. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out. The interior of the SPR is coated during production with purified CMV antigen. Each SPR is identified by the CMVM code.

Before each new lot of reagents is used, specifications must be entered into the instrument using the master lot entry card. Calibration, using the standart provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data has been entered. Calibration should than be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variation in assay signal.

One positive control and one negative control are included in each VIDAS CMVM kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. Results can not be validated if the control values deviate from the expected values.

Description of the CMVM Strip

The strip consist of 10 wells covered with a labelled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Specimen Type and Collection

Human serum collected in separating tube gel collected in the normal manner from the vein. Samples can be stored at 2-8°C for up to 5 days; if longer storage is required, freeze at -25° ± 6 C.

RESULTS

The CHORUS instrument expresses the result as an index (ratio between the OD value of the test sample and that of the cutoff) which can be used as a quantitative measure, as it is proportional to the amount of specific IgM present. The MINI-VIDAS instrument expresses the result as an index (ratio of the fluorescent signal found for the serum to be tested, over the standart signal stored in the memory.

Table 1: Interpretation of the Results

Cytomegalovirus IgM	ELISA (Chorus) (Index)	ELFA(Mini-Vidas) (Index)
Negative	<0.9	<0.7
Positive	>1.1	>0.9
Doubtful	0.9-1.1	0.7-0.9

The results for each technique are given in the tables below:

Table 2: The Results for ELFA and ELISA Techniques

Cytomegalovirus IgM	ELFA (Mini-Vidas)	ELISA (Chorus)
Negative	153	156
Positive	43	35
Doubtful	2	7

190 out of 198 samples (96%), gave compatible results. In particular, 153 samples gave negative results, 35 samples gave positive results and 2 samples gave doubtful results (table 2) with both techniques. It was observed that 3 samples were positive in Vidas instrument and negative in Chorus instrument and 5 samples were positive in Vidas instrument and doubtful in Chorus instrument (table 3).

Table 3: Details for Inconsistent Results

Positive (ELFA)	Doubtful (ELISA)	Positive (ELFA)	Negative (ELISA)
1.6	0.9	1.3	0.8
1.2	0.9	1.4	0.7
1.5	0.9	1.2	0.7
1.2	1.1		
1.5	1.1		

CONCLUSIONS

The CHORUS instrument expresses the result as an index (ratio between the OD value of the test sample and that of the cutoff) which can be used as a quantitative measure, as it is proportional to the amount of specific IgM present in the sample. The results of the assay must be interpreted with caution and in conjuction with information available from the clinical evaluation and other diagnostic data. Sera from patients in an early or late stage of the disease could give a repeatedly negative result close to the cut-off value. In such cases, a confirmation of the result is recommended. Also, all positive test results require careful interpretation since false positive reactions or heterotypic IgM responses may occur with sera from patients with heterophile – positive mononucleosis, or Varicella Zoster.

The MINI-VIDAS instrument expresses the result as an index (ratio of the fluorescent signal found for the serum to be tested, over the standart signal stored in the memory). Fluorescence is measured twice in the reagent strip reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced onto the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by substracting the background reading from the final result. Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted as part of a complete clinical profile. Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted as part of a complete clinical profile. Comparative evaluation of the two assays demonstrated a comparable sensitivity for all systems. Low specificity and sensitivity are the disadvantages of ELISA in CHORUS instrument. This happens because there is a non specific glycolipid antigen for *Cytomegalovirus*, which operates in a cross-reaction with antigens of different origins. Advantage of ELISA is measuring samples one by one (even a single analyse) and a short procedure time. ELFA technique showed a better ability to detect *Cytomegalovirus* IgM antibodies during the early stage of acute infection. Positive and negative samples produced a large difference in signal strength. Analysis of the results revealed a good level of concordance between the two assays and confirmed the usefulness of ELFA technique to diagnose acute cytomegalovirus infections.

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