Hayder Shkhair Obayes al-janabi et al /J. Pharm. Sci. & Res. Vol. 10(10), 2018, 2549-2550

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Comparative evaluation study of ELISA system and MINI-VIDAS system for detection of Cytomegalovirus IgM antibodies

Hayder Shkhair Obayes al-janabi, Ali Hamad Abed Kelkawi, Iman Fadhil Abdul-Hsin, Mohanad Jawad Kadhim*, Murtada M. Hussein

College of biotechnology, Al-Qasim Green University, Babel.

Abstract:

In this search an enzyme-linked immunosorbent assay (ELISA, applied in Biomerx ELISA instrument) and an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) have been compared to determine the most sensitive technique for the detection of Cytomegalovirus IgM antibodies.

There were 98 samples have been analyzed in each technique and 90 (91.8%) samples out of 98 gave compatible results. 72 samples gave negative results. 16 samples gave positive results and 2 samples gave equivocal results with both techniques. It was observed that 3 samples were positive in Mini-Vidas instrument and negative in ELISA instrument whereas 5 samples were positive in Mini-Vidas instrument and Indistinct in ELISA instrument. Comparative evaluation of the two assays demonstrated a comparable sensitivity for both systems. ELFA technique showed a better proficiency to detect Cytomegalovirus IgM antibodies during the early stage of acute infection and the results analysis of revealed a good level of agreement between the two assays and confirmed the usefulness of ELFA technique to diagnose of acute Cytomegalovirus infection.

INTRODUCTION

Cytomegalovirus (CMV) is a linear, double-stranded DNA virus with an icosahedral capsid, CMV also known as HHV-5 member of the Herpesviridae and is so named for the enlarged cells produced by active infections, these cells are characterized by the presence of foreign matter, especially in the nucleus (1).

Human cytomegalovirus (HCMV) is abundant pathogen in humans, infecting over 50 % of the world population (2). The virus is transmitted horizontally, vertically and via infected blood transfusions, also the transmission of the virus can occur via saliva, sexual contact, placental transfer, breastfeeding, solid-organ and hematopoietic stem cell transplantation (3). Despite of infection with CMV is generally asymptomatic in healthy children and adults , 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 central nervous system abnormalities (4).The diagnosis of acute maternal CMV infection usually achieved by the presence of immunoglobulin (IgM) and low-avidity IgG requires confirmation of fetal infection, which is typically performed using polymerase chain reaction (PCR) assays for CMV on amniotic fluid **(**5**)** Viral culture of the urine and saliva obtained within the first two weeks of life continues to be the gold standard for diagnosis of congenitally-infected infants (6). Serological diagnostic was developing by using several techniques .In this search an enzyme-linked immunosorbent assay (ELISA, applied in Biomerx ELISA instrument) and an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) have been compared to determine the most sensitive techniques for the detection of Cytomegalovirus IgM antibodies.

MATERIAL AND METHODS

Samples collection:

The 98 blood samples were collected from the vein of patient and handled with all precautions. The sera samples were separated by centrifuge at 1000 rpm for 5 minutes in tubes containing separating gel and were stored at 2-8°C for 2 days, or frozen for longer periods at -20°C. An enzyme-linked immunosorbent assay (ELISA) and an enzyme-linked fluorescent assay (ELFA , applied in Mini-Vidas instrument) were used in comparison with each other in their sensitivity for the detection of IgM antibodies in collected sera samples .

The Procedure of ELISA Technique:

This technique was carried out according to manufacture company (Biomerx/France) as follow: The partially purified Cytomegalovirus antigen was bound to the solid phase (surface of microwells). Diluted patient serum was added to wells through incubation with human serum diluted in a diluent which blocks the IgG so, if specific IgM antibody was exist ,it will bind to the viral antigen. All unbound materials are washed away . After washing step, the sample was incubated with the conjugate composed of monoclonal anti-human IgM antibodies labeled with peroxidase to bind it to the antibody-antigen complex. The unbound conjugate was eliminated and the peroxidase substrate(TMB Chromogenic Substrate) was added. The developed color was referred to the concentration of specific antibodies present in the serum.

The result was calculated as an index (the ratio between the optical density (OD) value of the test sample and that of the cut-off) which was a quantitative measure, as it was proportional to the amount of specific IgM present in the sample.

$$\text{Sample Positivity Index} = \frac{\text{OD of test sample}}{\text{cut-off value}}$$

The procedure of ELFA Technique

This technique was carried out according to manufacture company (Biomerx company-France) as follow: All steps of this assay were performed automatically by the instrument. The assay combines two step enzyme immunoassay sandwich method with a final fluorescent detection. Solid Phase Receptacle (SPR) (which is also serves as the pipetting device) was the container for binding of CMV IgM antibodies (if present in the serum of the sample) and the CMV antigen coating the anterior of the SPR. The unbound components were eliminated by the washing steps then sample was incubated with the conjugate composed of alkaline phosphatase-labeled monoclonal anti-human IgM antibody which was cycled in and out of the SPR. For remove unbound components a final wash step was done, the final detection step was adding the substrate (4-Methyl-umbelliferyl phosphate) and conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) and the fluorescence of which was measured at 450 nm. The results were automatically calculated by the instrument in relation to the calibration curve . Each calibration must also be checked using positive and negative control which were included in each VIDAS CMV kit since results cannot be validated if the control values were deviated from the expected values.

The result was calculated as an index (the ratio of the fluorescent signal found for the serum to be tested, over the standard signal stored in the memory

RESULTS AND DISCUSSION

According to the index of the CMV-IgM kit (Table-1).Ninety (90) samples out of 98 (90.8%) gave compatible results in both techniques . 72 samples gave negative results , 16 samples gave positive results and 2 samples gave equivocal results (Table-3).. It was observed that 5 samples were positive in Vidas instrument and negative in ELISA instrument and 3 samples were positive in Vidas instrument and Equivocal in ELISA instrument (Table-4).

Table 1: Interpretation of the Results

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

Table 2: The results of ELFA and ELISA Techniques

Cytomegalovirus IgM	ELISA		ELFA(Mini-Vidas)	
	NO.	%	NO.	%
Negative	75	76.5	72	73.5
Positive	16	16.3	24	24.5
Equivocal	7	7.1	2	2.04
Total	98		98	

Table -3: The compatible results of ELFA and ELISA Techniques.

Cytomegalovirus IgM	ELISA		ELFA (Mini-Vidas)	
	NO.	%	NO.	%
Negative	72	73.4	72	73.4
Positive	16	16.3	16	16.3
Equivocal	2	2.04	2	2.04
Total	90	(91.8%)	90	(91.8%)

Table 4: The incompatible results of ELFA and ELISA Techniques.

Cytomegalovirus IgM	ELISA		ELFA(Mini-Vidas)	
	NO.	%	NO.	%
Negative	3	3.06		
Positive			8	8.16
Equivocal	5	5.10		
Total	8	8.16	8	8.16

Table 5: Sensitivity and specificity for ELFA and ELISA Technique

Cytomegalovirus IgM	Sensitivity	Specificity
ELISA	**84.21**% (16/19*100)	100%
ELFA	100 %	100%

It's necessary to conform the result of new infected patients in an early and late stage of the disease since that the serum of patients may give a negative result close to the cut-off value (Equivocal result). As well as all positive results need careful interpretation since false positive reactions or heterotypic IgM responses may occur with sera from patients with heterophile–positive mononucleosis, or Varicella Zoster (7 and 8).

The result shows higher sensitivity in ELFA technique (100%) than ELISA technique (84.2 %) (Table 5) and this may be due to no specificity for glycolipid antigen of Cytomegalovirus, which operates in a cross-reaction with antigens of different origins, For this reason, assay results should be interpreted as part of a complete clinical profile. There a number of error sources in indirect ELISA when achieved on complete serum: (i) the presence of specific IgG leading to inhibition; (ii) the rheumatoid factor of the IgM class (IgM-RF) cause interference with IgG; and (iii) reaction of IgM antibody with host antigens present in serum (9). However ELISA was measuring samples one by one (even a single analysis) and short procedure time. In ELFA (Mini-Vidas) Fluorescence was 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) was calculated by subtracting the background reading from the final result. Interference may be encountered with certain sera containing antibodies directed against reagent components (10).

CONCLUSIONS

The results revealed a good level of accordance between the two assays and confirmed the usefulness of ELFA technique to diagnosis of acute cytomegalovirus infections.

REFERENCES

1. Aguayo, F.; Murayama, T. and Eizuru, Y. UL146 variability among clinical isolates of human cytomegalovirus from Japan. Biol. Res., 2010, 43(4), 475-480.
2. Pugel , E.,P., and Cekinovi, D. Pathogenesis of congenital cytomegalovirus infection of the central nervous system. Periodicum Biologorum , 2011, 113(1), 51–60.
3. Sia, I.G. and Patel,R. New strategies for prevention and therapy of cytomegalovirus infection and disease in solid-organ transplant recipients. Clin. Microbiol. Rev., 2000, 13, 83-121.
4. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer?. J. Intern.Med., 2006, 259(3), 219-246.
5. Buxmann, H.; Miljak, A.; Fischer, D.; Rabenau, H.F.; Doerr, H.Wand Schloesser R.L. Incidence and clinical outcome of cytomegalovirus transmission via breast milk in preterm infants 31 weeks. Acta .Paediatr., 2009, 98(2), 270–276.
6. Ross S.A.; Novak, Z. Pati, S.and Boppana, S.B. Overview of the diagnosis of cytomegalovirus infection. Infect. Disord. Drug. Targets., 2011, 11(5), 466-474.
7. Lazzarato T, Gabrielli L, Lanari M,. Congenital Cytomegalovirus Infection: Recent Advances in the Diagnosis of Maternal Infection. Hum. Immunol., 2004, 65, 410-415.
8. Cassaing , S.;Bessieres ,M. H.; Berry, A.; Berrebi, A.; Fabre R. and Magnaval, J. F. Comparison between two amplification sets for molecular diagnosis of toxoplasmosis by real time. PCR. J.Clin.Microbiol., 2006, 44, 720-772.
9. Revello, M. G. and Gerna, G. Diagnosis and Management of Human Cytomegalovirus Infection in the Mother, Fetus, and Newborn Infant. Clin. Microbi. Rev. 2002, 15(4), 680–715.
10. Blerta,L., and Anila, M. Preliminary evaluation of chorus system in comparison with Mini-vidas system for detection of Cytomegalovirus–IgM antibodies. Inter. J. of Res. in Appl., 2014, 2(1), 2321-2347.