RESEARCH ARTICLE **(Open Access)**

Comparison of ECL and ELISA immunoassays for toxoplasma and cytomegalovirus diagnosis in pregnant women

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Abstract

Aim of investigation: Comparison of an electrochemiluminescence technique (ECL, applied in Cobas 6000 instrument) and an enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument) for early diagnosis of Toxoplasma gondii and cytomegalovirus infections in pregnant women. Medical diagnostic is working to determine the most sensitive techniques required for diagnosis of these pathogens, in the framework of which is developed this scientific work. This is very important due to their multiple fetal infections during pregnancy.

Methods: ECL and ELISA techniques are used to detect anti-toxoplasma IgM and anti-cytomegalovirus IgM antibodies in pregnant women, during the first trimester of pregnancy. 200 samples were analyzed with both techniques and sensitivity and specificity are evaluated for each of them.

Results: ECL technique has resulted in higher sensitivity and specificity (98%-100%), while ELISA (CHORUS) technique has resulted in lower sensitivity and specificity (86.7%-97.3%).

Conclusion: Analysis Analysis of the results confirmed the usefulness of ECL technique for early diagnosis of Toxoplasma gondii and cytomegalovirus infections in pregnant women. Anyway, for diagnostic purposes, the results should always be assessed in conjuction with the patient's medical history and other clinical examinations.

Keywords: Electrochemiluminescence*; ELISA*; Cytomegalovirus; Toxoplasma gondii; sensitivity; specificity.

1. 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. Transmission of infection requires intimate contact with infected excretions such as saliva, urine, cervical and vaginal excretions, semen, breast milk and blood. 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 [31], [32], [35]. Toxoplasma gondii is a well-known obligate intracellular protozoa pathogen of virtually all warm-blooded animals and commonly infects human worldwide. The infection is mainly acquired by ingestion of food or water that is contamined by mature oocysts shed by cats or by undercooked meat containing tissue cysts. Acute infection of toxoplasmosis in early pregnancy of women carries the peril of transmitting the infection to the fetus with serious and unpredictable consequences in later life [34]. A first step in diagnosing acute primary congenital Cytomegalovirus and Toxoplasma infections is most commonly made by the detection of anti-CMV-specific IgG and IgM antibodies or anti-Toxoplasma-specific IgG and IgM antibodies. The assay of specific IgM is of great importance in the diagnosis of primary infection and samples being reactive for IgM antibodies indicate an acute, recent or reactivated infection [22], [36]. 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.

2. Materials and Methods

200 pregnant women, with age ranging from 18 to 40 years, who are retested for cytomegalovirus and toxoplasma IgM antibodies and have come out with a negative result in the preliminary testing, were involved in this sudy. Serum samples were analyzed with electrochemiluminescence assay (ECL, applied in COBAS 6000 instrument) and enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument), including 150 negative and 50 positive samples for anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies. Further, the results were used to build ROC curves and to calculate sensitivity and specificity (with SPSS and MedCalc), which are statistical measurements of quality of a test. In addition, these results are used to calculate the area under the ROC curve (AUC), which is a measure of how well a parameter can distinguish between two diagnostic assays.

2.1. Principle Electrochemiluminescence technique for detection of anti-cytomegalovirus IgM antibodies

This technique is applied on Cobas 6000 instrument. The test principle is µ-Capture with a total duration of 18 minutes. **The first incubation:** 10 µL of sample are automatically prediluted 1:20 with Elecsys Diluent Universal. Biotinylated monoclonal anti-h-IgM-specific antibodies are added.

The second incubation: CMV-specific recombination antigen labeled with a ruthenium complex and streptavidin-coated microparticles are added. Anti-CMV IgM antibodies present in the sample react with the ruthenium-labeled CMV-specific recombination antigen. The complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by CMV IgM calibration.

Description of the reagent: M: Streptavidin-coated-microparticles (transparent cap), 1 bottle, 6.5mL. R1: Anti-h-IgM-Ab-biotin (gray cap), 1 bottle, 9 mL. R2: CMV-Ag-Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 9 mL.

Specimen type and collection: Human serum collected in separating tube gel. Samples can be stored at 2-8°C for up to 5 days; if longer storage is required, freeze at -25° ± 6 C

2.2. Principle of Electrochemiluminescence technique for detection of anti-toxoplasma IgM antibodies

This technique is applied on Cobas 6000 instrument. The test principle is µ-Capture with a total duration of 18 minutes. **The first incubation:** 10 µL of sample are automatically prediluted 1:20 with Elecsys Diluent Universal. T. gondii-specific recombination antigen labeled with a ruthenium complex is added. Anti-Toxo IgM antibodies present in the sample react with the ruthenium-labeled T. gondii -specific recombination antigen.

The second incubation: Biotinylated monoclonal anti-h-IgM-specific antibodies and streptavidin-coated microparticles are added. The complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with

the signal of the cutoff value previously obtained by Toxo IgM calibration.

Specimen type and collection: Human serum collected in separating tube gel. Samples can be stored at 2-8°C for up to 5 days; if longer storage is required, freeze at -25° ± 6 C.

Description of the reagent: M: Streptavidin-coated-microparticles (transparent cap), 1 bottle, 6.5mL. R1: Toxoplasma-Ag-Ru(bpy)$_3^{2+}$ (gray cap), 1 bottle, 9 mL. R2: Anti-h-IgM-Ab-biotin (black cap), 1 bottle, 9 mL.

2.3.Principle of enzyme-linked immunosorbent assay for detection of anti-cytomegalovirus IgM antibodies

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 peroxidase. 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:

Position 1: Empty well in which the operator must place the undiluted serum.

Position 2: Conjugate 0,35 ml.

Position 3: Diluent for the samples 0,35ml.

Position 4: TMB substrate 0,35 ml.

Position 5: Uncoated microplate well.

Position 6: Microplate well coated with purified Toxoplasma antigens.

Position 7: Empty

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.

2.4. Principle of enzyme-linked immunosorbent assay for detection of anti-cytomegalovirus IgM antibodies

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 peroxidase. 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:

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Position 3: Diluent for the samples 0,35ml.

Position 4: TMB substrate 0,35 ml.

Position 5: Uncoated microplate well.

Position 6: Microplate well coated with purified Toxoplasma antigens.

Position 7: Empty

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

3. Results and Discussion

Results of anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies measurements are presented in summary in table 1.

Table 1: Results of ECL and ELISA immunoassays for detection of anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies.

Samples	ECL (COBAS 6000) CMV M		
	Positive	Doubtful	Negative
(Positive) N=50	49	0	1
(Negative) N=150	2	0	148
	ELISA (CHORUS) CMV M		
(Positive) N=50	39	5	6
(Negative) N=150	5	5	140
	ECL (COBAS 6000) TOXO M		
(Positive) N=50	50	0	0
(Negative) N=150	1	1	148
	ELISA (CHORUS) TOXO M		
(Positive) N=50	35	10	5
(Negative) N=150	4	2	144

Results of sensitivity and specificity of ECL and ELISA immunoassays for detection of anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies measurements are presented in summary in table 2.

Table 2: Results of sensitivity and speificity of ECL and ELISA immunoassyas for detection of anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies.

Assay	Sensitivity (%)	Specificity (%)
ECL (MINI-VIDAS) CMV M	98	98.7
ELISA (CHORUS) CMV M	86.7	96,6
ECL (MINI-VIDAS) TOXO M	100	99.3
ELISA (CHORUS) TOXO M	87.5	97.3

The evaluation of the results showed a good concordance between the two immunoassays: r=0,863 ($p< 0.01$) for the detection of anti-cytomegalovirus IgM antibodies and r=0,839, ($p< 0.01$) for the detection of anti-toxoplasma IgM antibodies. These results are expressed in the following scatterplots (Fig. 1, and Fig. 2,). Also, ANOVA analyse showed a non-signifikative difference between the two

immunoassays: F0.05[1,398] = 0,168, p = 0682 (α=0.05) for the detection of anti-cytomegalovirus IgM antibodies and F0.05[1,398] = 0,665, p = 0.415 (α=0.05) for the detection of anti-toxoplasma IgM antibodies.

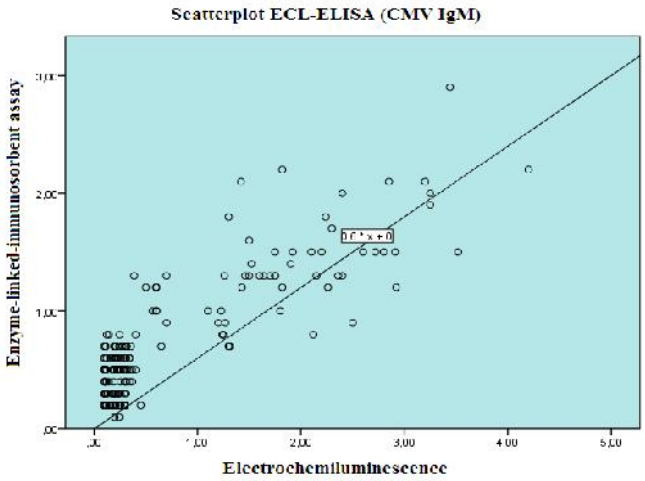

Figure 1. Scatterplot analysis of 200 sera measured by electrochemiluminescence assay (ECL-IgM) and enzyme-linked immunosorbent assay (ELISA-IgM) for detection of anti-cytomegalovirus IgM antibodies. The linear line is characterized by y=0,6*x equation. The scatterplot is nonlinear, positive with few values that deviate the linear line. This means that ECL and ELISA immunoassays have a good concordance for detection of anti-cytomegalovirus IgM antibodies.

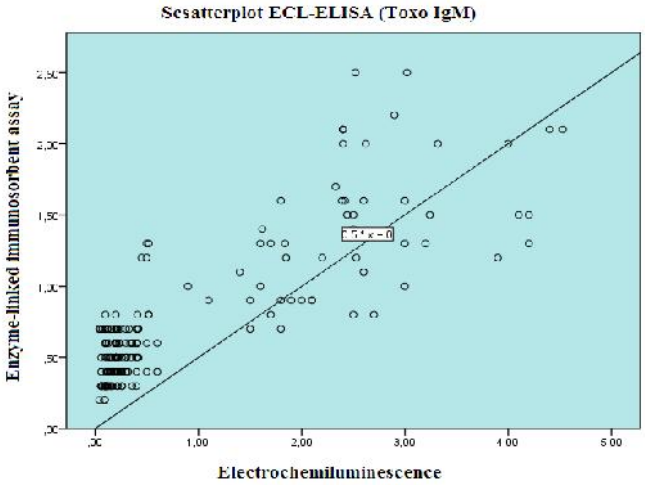

Figure 2. Scatterplot analysis of 200 sera measured by electrochemiluminescence assay (ECL-IgM) and enzyme-linked immunosorbent assay (ELFA-IgM) for detection of anti-toxoplasma IgM antibodies. The linear line is characterized by y=0.5*x equation. The scatterplot is linear, positive with few values that deviate the linear line. This means that ECL and ELISA immunoassays have a good concordance for detection of anti-toxoplasma IgM antibodies.

A receiver operating characteristics (ROC) curve was generated for each antibody tested (Fig. 3, and Fig.4,).

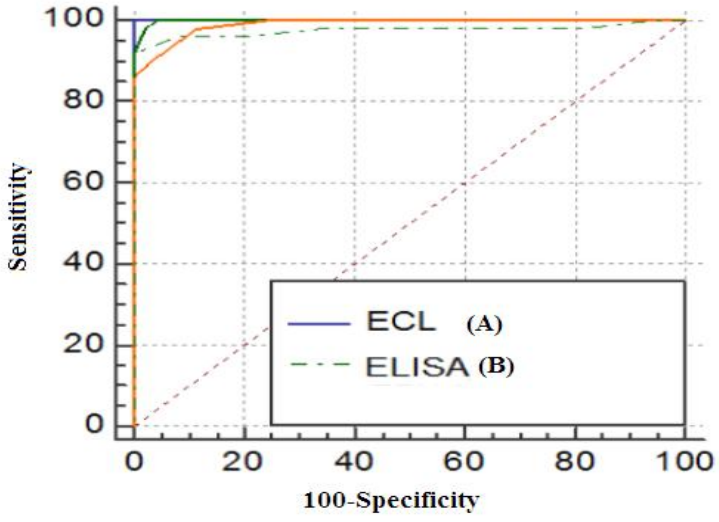

Fig 3. ROC Curves for comparison of electrochemiluminescence assay and enzyme-linked-immunosorben assay for detection of anti-cytomegalovirus IgM antibodies. (**A**) Shows the ROC curve of

electrochemiluminescence assay with an area under the ROC curve (AUC) of 1,00. (**B**) Shows the ROC curve of enzyme-linked-fluorescent assay with an AUC of 0.976.

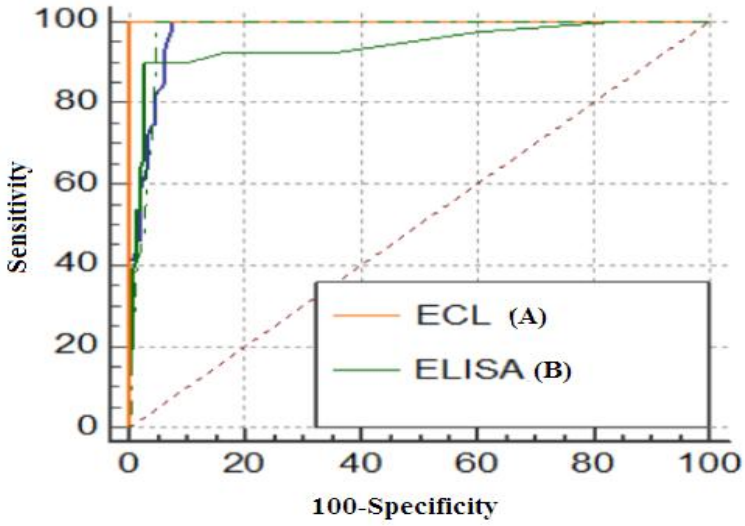

Fig 4. ROC Curves for comparison of electrochemiluminescence assay and enzyme-linked-immunosorbent assay for detection of anti-toxoplasma IgM antibodies. (**A**) Shows the ROC curve of electrochemiluminescence assay with an area under the ROC curve (AUC) of 1,00. (**B**) Shows the ROC curve of enzyme-linked-fluorescent assay with an AUC of 0.941.

The ROC curve is a fundamental tool for diagnostic test evaluation. In a ROC curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points of a parameter. Each point represents a sensitivity/specificity pair corresponding to a particular decision threshold [23]. The ROC area for electrochemiluminescence assay was higher than those for enzyme-linked-immunosorbent assay. No statistically significant differences in ROC curves were noted between electrochemiluminescence assay and enzyme-linked-immunosorbent assay for detection of anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies in pregnant women.

The two imunoassays resulted with AUC> 0.5. The ECL immunoassay resulted with high AUC (1,000) for the detection of anti-cytomegalovirus IgM antibodies and anti-toxoplasma IgM antibodies, while ELISA immunoassay resulted with lower AUC (0,976) for the detection of anti-cytomegalovirus IgM antibodies and 0,941 for the detection of anti-toxoplasma IgM antibodies. These results are expressed in ROC curves, (Fig. 3, and Fig.4,).

The ECL immunoassay resulted with high sensitivity and specificity of 98% and 98,7%, respectively, for the detection of anti-cytomegalovirus IgM antibodies and 100% and 99,3 %, respectively, for the detection of anti-toxoplasma IgM antibodies. These high values of sensitivity and specificity of the COBAS 6000 system and ECL technique, are attributed to some important features of this system: the use of two-dimensional barcode on all reagents to minimize possible errors and maintaining constant control of the calibration curve.

The ELISA immunoassay resulted with high sensitivity and specificity of 86,7% and 96,6%, respectively, for the detection of anti-cytomegalovirus IgM antibodies and 87,5% and 97,3%, respectively, for the detection of anti-toxoplasma IgM antibodies. 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[31], [36], [38].

In the diagnosis of pregnant women, especially in the first trimester of pregnancy, lack of false negative results for IgM antibodies to *Cytomegalovirus* and *Toxoplasma gondii* is very important. This relates to the fact that the existence of false negative results is associated with lack of diagnosis and treatment of acute infections in the fetus and newborn baby [31], [36], [38].

Fals positive, fals negative and doubtful results are related to the fact that samples in the initial phase of an acute infection, may not show detectable levels of anti- cytomegalovirus IgM and anti-toxoplasma IgM antibodies. For this reason the detection of antibodies against *Cytomegalovirus* and *Toxoplasma gondii* in a single sample is not enough [37] [38]. Also, interference may occur in some samples containing antibodies against components of the reagents, or in some patients specific anti-cytomegalovirus IgM and anti-toxoplasma IgM antibodies may return to nonreactive levels within weeks after infection with cytomegalovirus[37] [38].

Another reason may be the fact that there is a non specific glycolipid antigen for *Toxoplasma gondii,* which operates in a cross-reactionwith antigens of different origins, resulting in fals positive, fals negative and doubtful results [36]. Also samples of patients who suffer from primary infections of Epstein-Barr virus may result positive for anti-cytomegalovirus IgM antibodies. This may be related to the fact that both viruses are in the same family of herpesviruses [38].

4. Conclusion

In conclusion, analysis of the results revealed a good level of concordance between the two assays and confirmed the usefulness of electrochemiluminescence assay to diagnose acute Cytomegalovirus and Toxoplasma gondii infections in pregnant women.

5. References

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