A COMPARATIVE ANALYSIS ON THREE DIFFERENT FULLY AUTOMATED ANTI-TOXOPLASMA IGM IMMUNOASSAYS

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Abstract

Toxoplasma gondii is a parasitic protozoa which can be transmitted by eating infected meat or from mother to fetus during the first trimester of pregnancy. This microscopic parasit can cause fetal infection with unpredictable consequences in later life. Medical diagnostic is working to determine the most sensitive techniques for the detection of T. gondii antibodies, in the framework of which is developed this scientific work. An enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument), an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) and a new Electrochemiluminescence technique (ECL, applied in Cobas 6000 instrument) have been compared with each other for the detection of Toxoplasma IgM antibodies. There have been analyzed 50 patients with each technique. ELISA technique showed a specificity of 83% and a sensitivity of 95%, ELFA technique showed a specificity of 90% and a sensitivity of 97.5%, ECL showed a specificity of 100% and a sensitivity of 100%. Comparative evaluation of the three assays demonstrated a comparable sensitivity for all systems. Electrochemiluminescence technique showed a better ability to detect Toxoplasma IgM antibodies during the early stage of acute infection. Analysis of the results revealed a good level of concordance between the three assays in term of sensitivity and specificity, and confirmed the usefulness of Electrochemiluminescence technique to diagnose acute toxoplasmosis during the first trimester of pregnancy.

Keywords: *Electrochemiluminescence, ELISA, ELFA, Toxoplasma IgM.*

Introduction

Toxoplasma gondii is a well-known obligate intracellular protozoan 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. Medical diagnostic is working to determine the most sensitive techniques for the detection of T. gondii antibodies, in the framework of which is developed this scientific work. The detection of Toxo IgM antibodies is presumptive of an acute, recent or reactivated Toxoplasma infection.

Materials and methods

An enzyme-linked immunosorbent assay (ELISA, applied in CHORUS instrument), an enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument) and a new Electrochemiluminescence technique (ECL, applied in Cobas 6000 instrument) have been compared with each other for the detection of Toxoplasma IgM antibodies. There have been analyzed 50 patients with each technique. The serums of patients were collected using tubes containing separating gel.

Principle of ELISA technique: This technique is applied on CHORUS instrument, which is a new device in medical diagnostics. The partially purified Toxoplasma antigen is bound to the solid phase. Through incubation with human serum diluted in a diluent which blocks the IgG, the specific IgMare 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 peroxidise substrate 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.

Principle of Electrochemiluminescence technique: This technique is applied on Cobas 6000 instrument. The test principle is µ-Capture with a total duration of 18 minutes. In the first incubation, *T. gondii* antigen labelled with a ruthenium complex is added and react with Anti-Toxo IgM antibodies present in the sample. In the second incubation, 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. Application of a voltage to the electrode than induces chemiluminescent emission which is measured by a photomultiplier.

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. The reaction medium is cycled in and out of the SPR several times. Anti Toxo IgM antibodies present in serum will bind to the Toxoplasma antigen coating the anterior of the SPR. Unbound components are eliminated during the washing steps. An Alkaline phosphatase-labeled monoclonal antihuman 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.

The Cobas 6000 analyzer automatically calculates the cutoff based on the measurement of Cal1 and Cal2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

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

Toxoplasma IgM	**ECL(Cobas 6000)**	**ELISA (CHORUS)**	**ELFA (Mini-Vidas)**
Negative	< 0.8 COI	<0.9	<0.7
Positive	>1 COI	>1.1	>0.9
Doubtful	0.8-1 COI	0.9-1.1	0.7-0.9

Results

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

Table 2. The results for ECL technique

Toxoplasma IgM	**ECL(Cobas 6000)**
Negative	10
Positive	40
Doubtful	-

Table 3. The results for ELISA technique

Toxoplasma IgM	ELISA (CHORUS)
Negative	12
Positive	38
Doubtful	-

Table 4. The results for ELFA technique

Toxoplasma IgM	ELFA (Mini-Vidas)
Negative	11
Positive	39
Doubtful	-

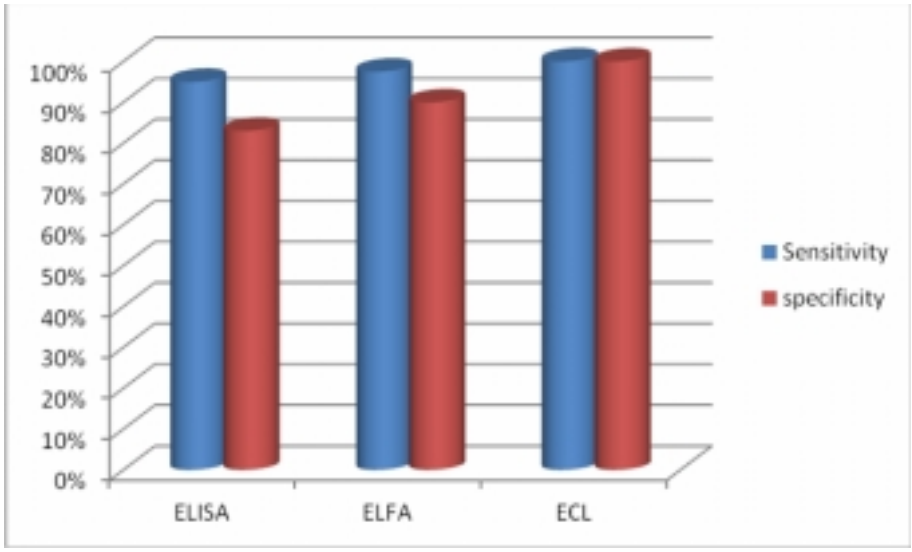
Based on the results, sensitivity and specificity are calculated for each technique:

ELISA technique showed a specificity of 83% and a sensitivity of 95%.

ELFA technique showed a specificity of 90% and a sensitivity of 97.5%.

ECL technique showed a specificity of 100% and a sensitivity of 100%.

Chart 1: Evaluation of sensitivity and specificity for ECL, ELISA and ELFA technique.



Conclusions

Comparative evaluation of the three 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 *Toxoplasma gondii*, 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 and ECL advantages in comparison with ELISA are high sensitivity and specificity. But, Electrochemiluminescence technique showed a better ability to detect Toxoplasma 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 three assays in term of sensitivity and specificity, and confirmed the usefulness of Electrochemiluminescence technique to diagnose acute toxoplasmosis during the first trimester of pregnancy.

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