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Evaluation of the Roche Elecsys Toxo IgG and IgM electrochemiluminescence immunoassay for the detection of gestational *Toxoplasma* infection

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Abstract

Unidentified gestational infection with *Toxoplasma gondii* may lead to fetal infection with severe complications later in childhood. Because diagnosis of maternal infection solely depends on serology, routine tests with high sensitivity and specificity are required. In this study, the new Roche Elecsys Toxo IgG and IgM immunoassay was compared with Sabin–Feldman dye test and immunosorbent agglutination assay-IgM as reference test. Serum samples were analyzed from 927 pregnant women, including 100 negative, 706 chronic, and 121 acute infections. The combination of both Elecsys IgG and IgM assays demonstrated high sensitivity and specificity of 97.1% and 100.0%, respectively, and a positive and negative predictive value of 100.0% and 81.3%, respectively. The Elecsys assay is a useful tool as a first-line screening method to detect gestational infections. However, if gestational infection is assumed, confirmatory testing by a reference laboratory might be necessary to discriminate between pre- and postconceptional infection to start antiparasitic treatment to avoid mother-to-fetus transmission and severe sequelae.

Keywords: *Toxoplasma gondii*; Congenital toxoplasmosis; Gestational infection; Serologic screening

1. Introduction

Unrecognized maternal postconceptional infection with the protozoan *Toxoplasma gondii* may cause a broad spectrum of clinical diseases, varying from subclinical infection to connatal toxoplasmosis with severe symptoms such as retinochoroiditis, intracerebral calcifications, hydrocephalus, and mental retardation (Montoya and Liesenfeld, 2004; Remington et al., 2000). Fetal infection risk increases with gestational age at maternal seroconversion, resembling a risk of 15% at 13 weeks, 44% at 26 weeks, and 71% at 36 weeks (Thiebaut et al., 2007). In contrary, severity of fetal damage after infection declines with gestational age. In the first trimester, infections mostly lead to abortions and may cause severe neurologic lesions within the second trimester. Within third trimester, infected fetuses very often present asymptomatically at birth (Wilson et al., 1980).

In Austria, pregnant women are subject to a nationwide routine serologic prenatal screening program. Initial *Toxoplasma* serology is carried out within the first trimester, mostly after the first visit at the gynecologist. In seronegative pregnant women, follow-up serology is recommended at 8-week intervals to identify seroconversions. Seropositive sera are further investigated to discriminate acute (postconceptional) from chronic (preconceptional) infection to avoid unnecessary retesting and, more important, to avoid unnecessary therapy. In the majority of laboratories, serologic screening is based on commercially available enzyme immunoassays, and every test system has to fulfill criteria of adequacy including high sensitivity and specificity, cost effectiveness, and easy handling under routine laboratory conditions.

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The Sabin–Feldman dye test (DT), still considered as the "gold standard" for the detection of *Toxoplasma* infection, is expensive and time consuming (Reiter-Owona et al., 1999). Its application is therefore restricted to specialized laboratories as confirmatory test and as standard for validation of new test systems.

In case of acute *Toxoplasma* infection, amniocentesis for direct fetal diagnosis by polymerase chain reaction (PCR) is performed (Gratzl et al., 1998; Kasper et al., 2009; Knerer et al., 1995) and antibiotic treatment according to the PCR result is recommended (Aspock et al., 1994). The transmission rate (Thiebaut et al., 2007) and dimension of sequelae depend on the period between seroconversion and initiation of treatment (Gras et al., 2004). Long serologic testing intervals are favoring fetal infection, whereas early prenatal treatment is able to reduce the risk of cerebral lesions and retinochoroiditis, the main complications of connatal toxoplasmosis (Gras et al., 2005; Kieffer et al., 2008).

The aim of the study was to evaluate the recently introduced Roche Elecsys Toxo IgG and IgM immunoassays on a Cobas 2010 system as a routine screening method for the detection of *T. gondii* antibodies in the sera of pregnant women. The Elecsys test results were compared with the DT results and the immunosorbent agglutination assay (ISAGA)-IgM for determination of anti–*T. gondii*-specific IgM.

2. Materials and methods

2.1. Serum specimens

A total of 927 blood samples from pregnant women were collected and assessed according to the recommendations of the Austrian Toxoplasmosis Screening Program by the toxoplasmosis laboratory located at the Department of Pediatrics and Adolescent Medicine, Medical University of Vienna. The discrimination of chronic and acute infection status was performed according to the criteria of Lebech et al. (1996) in A) no infection: DT negative; B) postconceptional (acute) infection: seroconversion during pregnancy; rising titer by DT; high titer by DT, ISAGA-IgM positive, and low IgG avidity in the third trimester; C) preconceptional (chronic) infection: low DT, negative/borderline ISAGA-IgM. The infection status was confirmed by serologic follow-up of the patients by DT and ISAGA-IgM.

2.2. Sabin–Feldman DT and ISAGA-IgM

The Sabin–Feldman DT was performed as described by Sabin and Feldman (1948). In brief, the test is based on complement-mediated cytolysis of antibody-coated live *T. gondii* tachyzoites, which is indicated by their ability to take up methylene blue (Reiter-Owona et al., 1999). The ISAGA-IgM was conducted as described by Desmonts et al. (1981). Results and interpretation of the ISAGA-IgM index are according to the manufacturer's recommendation for acquired *Toxoplasma* infections in adult and children: ISAGA index negative reaction in the range of 0 to 5, ISAGA borderline in the range of 6 to 8, ISAGA positive in the range of 9 to 12.

2.3. Roche Elecsys Toxoplasma IgG and IgM test system

An aliquot of the sera was analyzed by Roche Elecsys Toxo IgG and IgM assay (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. This automated system is based on an electrochemiluminescence immunoassay and is intended for use on Roche Elecsys and Roche "cobas e" immunoassay analyzers for the in vitro quantitative determination of IgG and IgM antibodies to *T. gondii* in human serum.

Both the Elecsys Toxo IgG and the Toxo IgM assay contain a recombinant native-like folded, immunologic fully active surface antigen 1 protein (SAG 1; formerly called p30) in a soluble form, thus, providing high reactivity. SAG 1 (p30) antigen used in the Toxo IgG assay has an exactly defined homodimeric structure, thus, preventing the binding of IgM antibodies, and only IgG (and additional IgA and IgE) are captured. A polymer SAG 1 (p30) antigen with multiple binding sites is used in the Toxo IgM assay, which allows the binding of IgM antibodies.

2.3.1. Elecsys Toxoplasma IgG assay

This involves the reaction of 10 μL of sample, a biotinylated recombinant *T. gondii*-specific antigen, and a *T. gondii*-specific antigen labeled with a ruthenium complex (Tris(2,2′-bipyridyl)ruthenium(II) complex ($Ru(bpy)_3^{2+}$)) forming a sandwich complex in the first incubation step. In the second step, streptavidin-coated microparticles are added and the complex binds to the solid phase by the interaction of biotin and streptavidin. The total duration of assay was 18 min at 37 °C reaction temperature on a Roche Elecsys system.

2.3.2. Elecsys Toxoplasma IgM assay

This assay is based on a μ-capture principle, and the total duration of assay was 18 min at 37 °C. In the first incubation, 10-μL sample is automatically prediluted in a ratio of 1:20 with Elecsys Diluent Universal. *T. gondii*-specific recombinant antigen labeled with a ruthenium complex is added. Anti-Toxo IgM antibodies present in the sample react with the ruthenium-labeled *T. gondii*-specific recombinant antigen. In the second step, biotinylated monoclonal h-IgM–specific antibodies and streptavidin-coated microparticles are added. The complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Finally, the separation of unbound and bound substances is performed in the electrochemiluminescence measuring cell (which is constructed as a flow chamber) by using a magnetic field holding the streptavidin-coated microparticles laden with immune complexes. Unbound reagent components and excess sample material are then removed from the measuring cell. Application of a defined voltage induces the electrochemiluminescent reaction, and the resulting light emission is measured directly by the photomultiplier. At the