

# MULTICENTER EVALUATION OF THE ELECSYS TOXO IgG AND IgM TESTS FOR THE DIAGNOSIS OF INFECTION WITH *TOXOPLASMA GONDII*

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Detection of IgG and IgM antibodies is commonly performed for the diagnosis of infection with *Toxoplasma gondii*. We determined the accuracy of the Elecsys Toxo IgG and IgM test at four European laboratories compared to local reference methods. Coefficients of variation for reproducibility ranged from 1.0 to 6.5% for IgG and from 0.8 to 3.2% for IgM. Seroconversion panels revealed high overall concordance with the reference tests. The Elecsys test detected IgG antibodies earlier than the Cobas Core IgG test in 19 of 47 panels; persisting IgM antibodies were observed in the VIDAS but not the Elecsys test in five of 47 panels. In 31.4% of latent stage sera with persistent IgM antibodies (positive LIASON IgM), the Elecsys IgM test gave negative results indicating increased “clinical” specificity. Sensitivity and specificity of the Elecsys IgG assay ranged from 99.45 to 100% and 87.50–99.80%, respectively, and 91.11–95.74 and 98.45–99.79% for the Elecsys IgM assay, respectively.

In conclusion, excellent reproducibility and accuracy make the Elecsys Toxo G and M tests highly suitable for the detection of anti-*T. gondii* IgG and IgM antibodies. The lower detection rates for persistent IgM in the Elecsys IgM test increase “clinical” specificity and decrease the need for follow-up testing.

**Keywords:** *Toxoplasma gondii*, antibodies, IgM, IgG, seroconversion, latent infection

## Introduction

Infection with the protozoan parasite *Toxoplasma gondii* typically does not result in signs or symptoms of the infection [1]. However, in rare cases, primary infection may lead to lymphadenitis or retinochoroiditis [1, 2]. Primary infection during pregnancy carries the risk of transmission of the parasite to the fetus, and the rate of transmission and severity of sequelae in the fetus are dependent on the age of gestation when infection is acquired [1]. Delays in diagnosis caused by long intervals between testing may result in fetal infection, whereas early prenatal treatment reduces the risk for development of cerebral lesions and retinochoroiditis [3, 4]. In immunocompromised patients, reactivation of the infection causes severe neurological damage and death if left untreated. In these patients, the presence of IgG but not IgM antibodies in-

dicates latent infection and identifies patients at risk for reactivation; in contrast, the detection of IgG and IgM antibodies is key for the diagnosis of acute infection in immunocompetent patients [1]. Enzyme-immunoassays for the detection of anti-*T. gondii* IgG and IgM antibodies have been developed with high clinical accuracy; in most laboratories, these tests are routinely used [5, 6].

The Elecsys Toxo IgG and IgM tests (Roche Diagnostics, Rotkreuz, Switzerland) are fully automated enzyme-immunoassays using chemiluminescence for the measurement of anti-*T. gondii* IgG and IgM antibodies [7–9]. The aim of this study was to validate the Elecsys Toxo IgG and IgM assays in four European laboratories using different panels of sera with known serological status as well as a large number of prospectively collected sera. Results indicate that the Elecsys Toxo IgG and IgM tests are highly reproducible and accurate allowing detection of *Toxoplasma* IgG and IgM antibodies.

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## Materials and methods

The present clinical study was performed to validate the accuracy of the Elecsys Toxo IgG and IgM tests. In addition to a reproducibility and cross-reactivity study, we performed clinical studies using seroconversion panels from patients with known serology status, and a large European multicenter study using prospectively collected consecutive sera as well as archived sera with known serology status.

### Reproducibility study

Reproducibility was determined using human sera, Elecsys reagents, and controls in a modified protocol of the National Committee for Clinical Laboratory Standards (NCCLS). Panels consisted of five members: human sera either negative ( $n = 1$ ) or positive ( $n = 2$ ) for anti-*T. gondii* antibodies and controls designed to give negative ( $n = 1$ ) to low positive ( $n = 1$ ) results. Within run and between run reproducibility were determined with six daily runs for 10 days.

### Potentially cross-reacting sera

A total of 455 potentially cross-reacting sera were tested with the Elecsys Toxo IgM and a comparator IgM test. Sera contained antibodies against HAV, HBV, HCV, HIV, CMV, EBV, HSV, VZV, rubella, *Treponema pallidum*, malaria, *Entamoeba histolytica*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*; autoantibodies (AMA, ANA); and elevated titers against rheumatoid factor, or were obtained after vaccination against HBV and influenza.

### Seroconversion panels and sera from patients with latent infection

Seroconversion panels were obtained from pregnant women in Marseille and Paris. Pregnant women had been tested under the French screening program for toxoplasmosis mandating screening of all pregnant women during the first trimester of pregnancy and monthly controls if the first serology is negative. Panels consisted of at least three and up to five consecutive sera obtained shortly before or early after infection. The stage of infection had been established using IgG and IgM Toxoplasma tests combined with tests for IgA and avidity and/or differential agglutination antibodies.

Sera obtained from patients during the latent stage of infection (>3 months) were used to evaluate the persistence of anti-*T. gondii* IgM antibodies compared to the Liason Toxo IgM test (DiaSorin, Saluggia, Italy). All sera had high avidity Toxoplasma IgG antibodies.

### Method comparison studies

Four European sites participated in the method comparison studies. All reference tests performed at the sites (Cobas Core Toxo, VIDAS Toxo, Advia Toxo, Platelia Toxo, and AxSym Toxo) were performed as recommended by the manufacturers.

A total of 492 samples were tested at the Toxoplasma Consulting Laboratory, Institute for Microbiology and Hygiene at the Charite Medical School in Berlin, Germany by the Elecsys Toxo G assay for *Toxoplasma*-specific IgG-antibodies compared to the VIDAS Toxo IgG assay (Biomérieux, Nürtingen, Germany). These included 247 repository samples from patients either uninfected (25), with remote (4), latent (182), or acute infections (34). A total of 496 sera were tested by the Elecsys Toxo M assay compared to the VIDAS Toxo IgM assay. At the CHUV in Lausanne, Switzerland, 250 routine sera and 250 repository sera from patients with remote (50), latent (50), or acute (50) infections as well as 50 sera with potentially cross-reactive antibodies were tested in the Elecsys Toxo G and M compared to the VIDAS Toxo IgG and IgM assays (Biomérieux).

Lastly, 40 frozen mother–child serum sets obtained at birth were analyzed to detect congenital infection using immunoelectrophoretic profiles (ELIFA). Overall, 120 specimens were available (between 1 and 5 specimens (average 2) from the newborns). Elecsys Toxo G and M results were compared to Toxoscreen DA IgG (BioMérieux) and ISAGA titers (BioMérieux), respectively.

In the Laboratory of Parasitology and Mycology at the Hôpital de la Timone in Marseille, France, 526 sera were tested using the Elecsys Toxo G assay compared to the Cobas Core Toxo IgG (Roche Diagnostics, Rotkreuz, Switzerland) assay; there were 200 routine sera and 326 repository sera from patients either uninfected (54), with remote (50), latent (52), or acute infections (50); 32 potentially cross-reactive sera were also included. IgM antibody results were compared in 526 sera between the Elecsys Toxo IgM and VIDAS Toxo IgM assay. In addition, 83 sera obtained from 25 seroconverting pregnant women were tested. A total of 508 sera were tested in Paris, France, using the Elecsys Toxo IgG assay compared to the Platelia Toxo IgG assay (Bio-Rad, Marnes-la-Coquette, France). There were 198 routine sera and 308 repository sera from patients being either uninfected (52), or with remote (50), latent (52), or acute infections (50); 13 potentially cross-reactive sera were also included. A total of 506 sera were tested using the Elecsys Toxo M and Platelia Toxo IgM assays. In addition, 73 sera from 24 pregnant women with seroconversions were tested.

### Resolution of discordant results

Predefined algorithms specific to the testing sites were used for resolution of discordant test results between the

Elecsys Toxo IgG and IgM and the reference tests. In all cases of discordant results, a repeat run of both tests was performed initially. If discordant results persisted, additional tests including the Sabin Feldman Dye test (considered the gold standard for detection of anti-*T. gondii* IgG antibodies [1, 10]), the highly sensitive direct agglutination test [11], and other commercially available enzyme immunoassays were used. The rule of majority was used to resolve discordant results at sites that performed more than two tests for the detection of anti-*T. gondii* IgG antibodies. To resolve discrepancies in IgM test results, additional IgM tests including IgM immunofluorescence test and IgG avidity assays [5], in addition to reviewing available clinical information, were used. All comparator and reference tests were performed as recommended by the manufacturers.

#### *Elecsys Toxo IgG and IgM assay*

The Elecsys Toxo IgG test is a quantitative sandwich test based on recombinant surface antigen SAG1 (p30). The sample volume is 10  $\mu$ l. The duration of the test is 18 min consisting of the following steps: 1) incubation of sample with biotinylated recombinant *T. gondii*-specific antigen and *T. gondii*-specific recombinant antigen labeled with a ruthenium complex; 2) incubation of streptavidin-coated microparticles; 3) magnetic capture, removal of unbound substances, chemiluminescent emission measured by a photomultiplier; and 4) determination of results via a calibration curve standardized against the 3rd International Standard (TOXM NIBSC, UK). The cut-off is 3 IU/ml; results  $\geq 1$ –2.9 IU/ml are considered indeterminate. No cross-reactivity was observed with sera positive for HIV, HBV, HCV, CMV, HSV, VZV, EBV, ParvoB19, rubella, chlamydia, malaria, and syphilis.

The Elecsys Toxo IgM test is a  $\mu$ -capture test based on polymeric structure of the recombinant surface antigen SAG1. Toxoplasma IgG antibodies are removed by capture to unlabeled monomer SAG1. The sample volume is 10  $\mu$ l. The duration of the test is 18 min consisting of the same steps as described above for the IgG test. The cut-off is 1.0; results  $>0.8$ –0.99 are considered indeterminate. No cross-reactivity was observed with sera positive for HIV, HAV, HBV, HCV, CMV, HSV, VZV, EBV, rubella, chlamydia, malaria, syphilis, AMA, and ANA.

#### *Statistical analyses*

Relative sensitivities and relative specificities including confidence intervals (CI) were calculated for the comparison of results after resolution of discrepancies. Samples with indeterminate results in any method were not included in the performance calculations.

## Results

#### *Reproducibility*

The coefficients of variation for within run and in between run reproducibility for the detection of IgG anti-*T. gondii* antibodies using human sera and controls ranged from 1.9 to 2.5% and 2.7 to 4.0%, respectively; the coefficients of variation for within run and in between run reproducibility for the detection of IgM anti-*T. gondii* antibodies ranged from 1.1 to 2.2% and 2.5 to 5.4%, respectively.

#### *Potentially cross-reacting sera*

Among 455 sera with potentially cross-reacting antibodies against a variety of infectious agents, rheumatoid factor, other autoantibodies or post-vaccination antibodies, the Elecsys Toxo IgM test gave positive results in four (AMA; malaria, EBV, VZV) of 455 cases. In two (AMA, VZV) of these cases, a comparator anti-*T. gondii* IgM test also gave positive results; three additional sera (AMA, malaria, anti-HBc IgM) also gave positive results in the comparator but not the Elecsys IgM test.

#### *Seroconversion panels*

Forty-seven seroconversion panels consisting of three to five sera each were tested in the Elecsys Toxo IgG and IgM tests compared to the Cobas Core IgG and VIDAS or Platelia IgM tests. Concordant results were observed in the majority of panels (28/47). Discordant results observed when comparing the IgG and IgM test results are listed in *Table 1*. In 19 of 47 panels with discordant results, IgG seroconversion was detected earlier in the Elecsys Toxo IgG compared to the comparator IgG tests. In 16 of 19 cases, the Elecsys Toxo IgG test gave an initial intermediate result while the Cobas Core ( $n = 8$  in Marseille) or Platelia IgG ( $n = 8$  in Paris) test remained negative until the following blood draw (performed between 1 and 8 weeks later); in three additional cases, the Elecsys test gave an initial positive result while the Cobas Core or Platelia IgG test remained negative until the following blood draw. We also observed higher IgG titers as expressed in IU/ml compared to the comparator IgG tests (data not shown). There was 100% agreement between results in the Elecsys Toxo IgM and VIDAS Toxo IgM tests (Marseille); in two of 22 panels (Paris), the Platelia IgM test gave a positive result while the Elecsys Toxo IgM test remained negative (a concordant positive result was observed in the follow-up sera obtained between 2 and 3 weeks later). Of interest, these two sera also tested initially negative with the VIDAS Toxo IgM test.

In five sera, we observed the disappearance of IgM antibodies in the Elecsys IgM test (4.5–36 weeks after seroconversion) whereas the VIDAS test continued to give positive results.

**Table 1.** Discordant results in seroconversion panels ( $n = 25$  in Marseille,  $n = 22$  in Paris) between Elecsys and comparator IgG and IgM tests

	Result constellation Elecsys/comparator	IgG		IgM	
		Marseille (Elecsys vs. Cobas Core)	Paris (Elecsys vs. Platelia)	Marseille (Elecsys vs. VIDAS)	Paris (Elecsys vs. Platelia)
Seroconversion (negative to positive)	-/+	0	0	0	2*
	±/-	8	8	0	0
	+/-	1	2	0	0
Disappearance of antibodies in f/up sera (positive to negative)	-/+	0	0	5**	0
	±/-	0	0	0	0
	+/-	0	0	0	0

\*VIDAS IgM also gave negative results in the same sera

\*\*After 4–36 weeks

These results point towards an increased sensitivity for the detection of IgG antibodies in the Elecsys compared to the Cobas Core and Platelia tests. In addition, the Elecsys IgM test appeared to show shorter persistence of IgM antibodies.

#### Sera with persistent anti-*T. gondii* IgM antibodies

A panel of 35 sera from subjects with latent infections (>3 months post infection as confirmed by the presence of high

IgG avidity antibodies) and positive IgM titers in the Liaison IgM test was tested with the Elecsys Toxo IgM and Cobas Core test (Table 2). Twenty-four of 35 sera gave concordant positive results between the Elecsys Toxo IgM and Liaison Toxo IgM test; however, in 11 sera, IgM antibodies were undetectable using the Elecsys Toxo IgM test. Elecsys Toxo IgM and Cobas Core IgM tests showed 23 concordant positive and nine concordant negative results. These results further underline the tendency towards shorter persistence of positive Elecsys IgM results high

**Table 2.** Detection of anti-*T. gondii* IgM antibodies in patients with latent Toxoplasma infection (>3 month post infection as indicated by the presence of high IgG avidity antibodies)

Serum no.	Liaison Toxo IgM (pos.: >10)	Elecsys Toxo IgM (pos.: >1.0, neg.: <0.8)	Cobas Core IgM (pos.: >100%, neg.: <90%)
1	11.5 (+)*	0.45	47.70
2	37.0 (+)	1.89 (+)	251.50 (+)
3	12.0 (+)	1.59 (+)	171.80 (+)
4	12.0 (+)	0.89	156.50 (+)
5	80.0 (+)	1.50 (+)	274.0 (+)
6	11.0 (+)	0.43	30.80
7	14.0 (+)	0.27	28.50
8	15.0 (+)	1.48 (+)	155.70 (+)
9	11.0 (+)	3.15 (+)	291.30 (+)
10	19.0 (+)	2.59 (+)	147.70 (+)
11	18.0 (+)	1.47 (+)	192.50 (+)
12	14.0 (+)	0.75	94.30
13	14.0 (+)	1.68 (+)	90.80
14	39.0 (+)	1.70 (+)	399.40 (+)
15	28.0 (+)	1.88 (+)	185.10 (+)
16	75.0 (+)	1.58 (+)	407.20 (+)
17	30.0 (+)	0.22	10.30
18	19.0 (+)	7.14 (+)	564.70 (+)
19	15.0 (+)	3.75 (+)	409.8 (+)
20	14.0 (+)	0.39	36.10
21	13.0 (+)	0.24	13.70

**Table 2.** (cont.)

Serum no.	Liaison Toxo IgM (pos.: >10)	Elecsys Toxo IgM (pos.: >1.0, neg.: <0.8)	Cobas Core IgM (pos.: >100%, neg.: <90%)
22	16.0 (+)	10.03 (+)	564.70 (+)
23	45.0 (+)	3.45 (+)	535.50 (+)
24	15.0 (+)	1.40 (+)	228.80 (+)
25	26.0 (+)	2.95 (+)	414.10 (+)
26	11.0 (+)	0.20	17.40
27	10.0 (+)	1.89 (+)	191.0 (+)
28	119.0 (+)	2.45 (+)	397.7 (+)
29	12.0 (+)	1.82 (+)	132.80 (+)
30	22.0 (+)	4.03 (+)	564.70 (+)
31	10.0 (+)	1.22 (+)	81.90
32	44.0 (+)	0.26	23.40
33	>150 (+)	5.78 (+)	564.70 (+)
34	14.0 (+)	3.94 (+)	519.80 (+)
35	42.0 (+)	0.30	31.90

\*(+), positive result

compared to comparator IgM tests indicating increased “clinical” specificity.

#### Method comparison studies

The multicenter method comparison study was performed at four European sites. Sites compared the performance of the Elecsys Toxo IgG and IgM tests to reference tests routinely performed in the respective laboratories. Results of the multicenter comparative study are presented by antibody class and site.

#### Comparison of IgG results

##### Berlin, Germany

A total of 498 sera were tested using the Elecsys Toxo IgG and the VIDAS Toxo IgG tests (Table 3). Among these, 358 (71.89%) showed concordant positive and 105

(21.08%) concordant negative results. There were 18 VIDAS negative/Elecsys positive, two VIDAS positive/Elecsys negative, four Elecsys grey zone/VIDAS negative, and 11 VIDAS grey/Elecsys positive test results. Among the Elecsys positive/VIDAS negative samples, three of 18 showed VIDAS positive results when retested and, thus, were considered concordant positive. After discordant testing, the relative sensitivity of the Elecsys Toxo IgG test was 99.45% (CI 98.02–94.45%) and the relative specificity was 87.5% (CI 80.22–92.83%) (Table 3).

##### Lausanne, Switzerland

Five hundred sera were tested in the Elecsys Toxo IgG and VIDAS Toxo IgG test. Results are shown in Table 3. A total of 219 (43.8%) sera gave concordant positive and 256 (51.2%) concordant negative results. There were nine VIDAS negative/Elecsys positive, one VIDAS positive/Elecsys negative, four Elecsys grey zone/VIDAS nega-

**Table 3.** Relative sensitivity and relative specificity of the Elecsys Toxo G test compared to commercially available reference IgG assays before and after resolution of discordant results

Site	Comparator assay	IgG before resolution		IgG after resolution	
		Sensitivity (CI) in %	Specificity (CI) in %	Sensitivity (CI) in %	Specificity (CI) in %
Berlin	VIDAS	99.44 (97.51–99.98)	85.37 (79.77–92.49)	99.45 (98.02–94.45)	87.5 (80.22–92.83)
Lausanne	VIDAS	99.55 (97.49–99.99)	96.6 (93.65–98.44)	99.55 (97.49–99.99)	99.49 (96.18–99.59)
Marseille	Cobas Core	99.29 (97.45–99.9)	96.55 (93.32–98.5)	100 (98.69–100)	99.57 (97.61–99.99)
Paris	Platelia	99.56 (97.58–99.99)	94.49 (90.92–96.95)	100 (n.d.)	100 (n.d.)

n.d., CI not determined. For specific results of discrepant testing, see the Results section of the article

tive, and 11 VIDAS grey zone/Elecsys positive, negative, or grey zone results. Among nine Elecsys positive/VIDAS negative samples, one sample gave VIDAS positive results upon testing, four samples were confirmed positive by the Toxoscreen direct agglutination assay, and in three sera, the initial VIDAS negative result was confirmed; no additional information was available for one serum. One VIDAS positive and Elecsys negative result was confirmed as true positive. Thus, the relative sensitivity of the Elecsys Toxo IgG compared to the VIDAS test was 99.55% (CI 97.49–99.99%) and the relative specificity was 99.49% (CI 96.18–99.59%) (Table 3).

Regarding mother–child pairs, concerning IgG, there was overall good agreement between the Elecsys Toxo IgG test and the comparator tests. One major discrepancy (positive by Elecsys Toxo IgG but negative by DA) was observed in a maternal sample; however, the titers in the Elecsys Toxo IgG test were 5.44 IU/ml (barely above cut off) and 4 IU/ml (negative) in the DA test. We also observed six minor discrepancies (positive Elecsys Toxo IgG vs. grey zone DA test, or grey zone Elecsys Toxo IgG vs. negative DA test) among newborn samples. These results point towards a superior sensitivity of the Elecsys Toxo IgG test compared to DA reference test. This is further supported by the observation that IgG in passively immunized children was detected up to 3 months longer in the Elecsys Toxo IgG compared to the DA test (data not shown).

#### Marseille

A total of 526 sera were tested in the Elecsys Toxo IgG and Cobas Core Toxo IgG tests (Table 3). There were 279 (53.04%) concordant positive, 224 (42.59%) concordant negative, eight Elecsys positive/Cobas Core negative, two Elecsys negative/Cobas Core positive, three Cobas Core positive/Elecsys grey zone, and ten Elecsys grey zone/Cobas Core negative results. In ten sera with discordant results, repeat testing ( $n = 3$ ) or reference testing using the Sabin Feldman dye test or the highly sensitive direct agglutination assay confirmed the Elecsys Toxo IgG positive/Cobas Core Toxo IgG negative results. Relative sensitivity was 100% (CI 98.69–100%), and relative specificity was 99.57% (CI 97.61–99.99%) (Table 3).

#### Paris

Among 506 sera tested with the Elecsys Toxo IgG and the Bio-Rad Platelia Toxo IgG test, 227 (44.86%) sera gave concordant positive results and 240 (47.43%) sera gave concordant negative results (Table 3). There were 14 Elecsys positive/Platelia negative and one Elecsys negative/Platelia positive tests; in all cases, the positive result was confirmed as true positive using indirect immunofluorescence. Thirteen Platelia negative/Elecsys grey zone, eight Platelia grey zone/Elecsys positive, and three Platelia grey zone/Elecsys grey zone tests were observed; in all cases, Elecsys results were confirmed as true positive or indicative of seroconversion. The relative sensitivity of the Elecsys Toxo IgG test compared to the Platelia Toxo IgG test

was 99.56% (CI 97.58–99.99%), and the relative specificity was 94.49% (CI 90.92–96.95%) (Table 3).

#### Comparison of IgM results

##### Berlin

A total of 496 sera were used to compare the Elecsys and VIDAS Toxo IgM tests (Table 4). About 376 (75.81%) concordant negative and 67 (13.51%) concordant positive results were observed among 496 sera. There were four VIDAS negative/Elecsys positive, 23 VIDAS positive/Elecsys negative, and 26 sera with grey zone results. After resolution of discordant results, the relative sensitivity was 91.11% (CI 83.23%–96.08%) and the relative specificity was 99.21% (CI 97.71–99.84%) (Table 4).

##### Lausanne

Among 500 sera tested with the VIDAS Toxo IgM and Elecsys Toxo M tests, we observed 381 (76.2%) concordant negative and 83 (16.6%) concordant positive results; six sera were VIDAS negative/Elecsys positive, 11 were VIDAS positive/Elecsys negative, and 19 gave grey zone results (Table 4). IgM immunofluorescence test results in all but one VIDAS positive/Elecsys negative sample were negative; ISAGA IgM was very low positive or even negative. In four cases, an acute infection was diagnosed while, in the other seven cases, the VIDAS Toxo IgM most likely detected persisting IgM antibodies turning the negative Elecsys Toxo IgM results a true negative result. Following resolution of discrepant results, the relative sensitivity was 95.74% (CI 89.46–98.83%) and the relative specificity was 98.45% (CI: 96.66–99.43%) (Table 4).

In mother–child paired specimens, we observed three major and nine minor discrepancies (data not shown). In maternal sera, all discrepancies indicated a lower sensitivity of Elecsys Toxo IgM test compared to ISAGA. In newborn sera, there were four major discrepancies also pointing towards a decreased sensitivity of the Elecsys Toxo IgG test compared to ISAGA; congenital infection in three of four newborns was confirmed by the presence of IgA antibodies, positive immune-electrophoresis profiles by ELIFA, or the presence of Toxoplasma DNA in amniotic fluid.

##### Marseille

Comparing the Elecsys Toxo M with the VIDAS Toxo IgM tests on 526 sera in Marseille, we found 334 (63.5%) concordant negative and 143 (27.19%) concordant positive results (Table 4). Four samples showed VIDAS negative/Elecsys positive, 21 VIDAS positive/Elecsys negative, and 24 grey zone results. After resolution, relative specificity was 99.11% (CI 97.43–99.82%) and relative sensitivity was 93.13% (CI 88.03–96.52%) (Table 4).

##### Paris

A total of 506 sera were tested in a method comparison between the Platelia Toxo IgM and Elecsys Toxo IgM test

**Table 4.** Relative sensitivity and relative specificity of the Elecsys Toxo M test compared to commercially available IgM assays before and after resolution of discordant results using reference tests

Site	Comparator assay	IgG before resolution		IgG after resolution	
		Sensitivity (CI) in %	Specificity (CI) in %	Sensitivity (CI) in %	Specificity (CI) in %
Berlin	VIDAS	74.44 (64.16–83.06)	98.95 (97.33–99.71)	91.11 (83.23–96.08)	99.21 (97.71–99.84)
Lausanne	VIDAS	88.30 (80.03–94.01)	98.45 (96.66–99.43)	95.74 (89.46–98.83)	98.45 (96.66–98.43)
Marseille	VIDAS	87.20 (81.09–91.9)	98.82 (97.0–99.68)	93.13 (88.03–96.52)	99.11 (97.43–99.82)
Paris	Platelia	88.69 (82.9–93.05)	99.69 (98.3–99.99)	95.51 (90.97–98.18)	99.69 (98.3–99.9)

in Paris (Table 4). About 325 (64.23%) sera tested concordant negative, and 149 (29.45%) sera tested concordant positive; there were one Platelia negative/Elecsys positive and 19 Platelia positive/Elecsys negative sera; of 12 grey zone results in the Elecsys test, eight were positive and four were negative in the Platelia IgM test. After resolution, the relative sensitivity was 95.51% (CI 90.97–98.18%) while the relative specificity was 88.69% (CI: 82.9–93.05%) (Table 4).

## Discussion

Depending on the patient's immune status and on the disease setting, the diagnosis of infection with *T. gondii* can be established with a variety of techniques. Serological tests are performed to determine the immune status in pregnant women and in organ donors or transplant recipients [1, 5, 12]. In patients with lymphadenopathy, uveitis, or retinochoroiditis and in newborns, serologic testing is used to diagnose toxoplasmosis [1, 5]. Whereas some European countries including France have a policy for the prevention of congenital toxoplasmosis using systematic serologic screening of pregnant women, other countries, i.e., Switzerland, have discontinued such screening programs [13]. In France, a first serology is performed before the end of the first trimester of pregnancy, and in IgM-positive cases, further testing is performed to establish the diagnosis of acute infection resulting in treatment. In the case of a negative result, serologic testing is repeated every month until delivery. For screening and diagnostic testing in subjects with suspected infection with *T. gondii*, serological tests need to demonstrate a balance between sensitivity and specificity; the interpretation of results also depends on the clinical setting [5].

In the present study, we investigated the accuracy of the Elecsys Toxo IgG and IgM tests using seroconversion panels and selected sera from subjects with latent infections. Furthermore, in a clinical trial at four European sites, a large number of banked sera with known results and prospectively collected routine sera were included. To compare results in a “real world” scenario, each site com-

pared results of the Elecsys tests with IgG and IgM tests routinely performed in the laboratory. Additional testing was performed to determine the true status of the subject in case of discordant results.

The Elecsys Toxo IgG and IgM tests demonstrated excellent accuracy compared to other IgG and IgM tests in all subsets of sera tested. A trend towards increased sensitivity for the Elecsys Toxo IgG test was observed in the seroconversion panels since earlier seroconversion from negative to equivocal or from negative to positive was observed compared to the Cobas Core and Platelia Toxo IgG tests. The sensitivity and specificity for the detection of anti-*T. gondii* IgG antibodies ranged from 99.29 to 99.56% and were similar at the four sites. We also observed higher IgG titers as expressed in IU/ml compared to the comparator IgG tests (data not shown). Increased sensitivity in clinical settings is of importance since it may translate into earlier diagnosis of pregnant women in screening programs allowing for earlier treatment that is associated with improved outcome [3–5]; in addition, increased sensitivity in other clinical settings allows to diagnose and manage infection in more subjects [5]. Conversely, increased sensitivity may result in prolonged detection of passively transferred maternal IgG antibodies in newborns when using the Elecsys Toxo IgG.

The increased sensitivity for anti-*T. gondii* IgG antibodies observed in the present multicenter study is in line with earlier report from smaller evaluations. Jost et al. [14] used immunoblotting to compare results in the Elecsys Toxo IgG and Platelia IgG test in pregnant women known to have seroconverted based on monthly monitoring. Among initial sera with IgM-positive results, 7.7% gave equivocal (92.3% negative) results for IgG in the Platelia IgG test compared to 69.2% equivocal (30.8% negative) IgG results in the Elecsys Toxo test. These results mirror results observed using seroconversion panels in the present study and underline the high sensitivity of the Elecsys Toxo IgG test. When comparing to the Sabin–Feldman dye test, a 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, were observed in an Austrian cohort of 927 pregnant women [7]. Thus, the

Elecsys Toxo IgG and IgM tests are useful tools for first-line screening of infection with *T. gondii*. Van Helden [8] reported a sensitivity of 100% and a specificity of 99.91% for the Elecsys Toxo IgG test in a cohort of 2936 clinical routine samples compared to the ADVIA Centaur Toxo IgG test. Increased sensitivity of the Elecsys Toxo IgG test compared to comparator tests may result in anxiety when discordant results are observed. A newly developed neutralization test [15] and a commercially available Western Blot [9] were used to resolve discrepancies; in both studies, the vast majority of Elecsys Toxo IgG positive/comparator IgG negative results were confirmed as true positive.

The sensitivity of the Elecsys Toxo IgM test was comparable to the comparator tests ranging from 99.45 to 100%. The specificity ranged from 87.5 to 99.57%; of interest, the markedly lower specificity of 87.5% was only observed at one site. Decreased specificity for IgM (and IgG) in Berlin compared to other sites is most likely caused by the type of laboratory (academic laboratory with reference laboratory certification and comparably low number of “clinical routine” sera); such differences in accuracy when testing “clinical routine” sera vs. sera submitted for reference testing have previously been reported by us [16].

Prusa et al. [7] compared the Elecsys IgM assay to the infection status and reported a sensitivity of 94.1%; the specificity was 79.0%, and the positive predictive and negative predictive values were 40.7 and 98.9%, respectively. Of interest, the seroconversion panels in the present study revealed two Elecsys Toxo IgM negative/comparator IgM positive sera early. These results are of concern in a clinical setting since a delay in diagnosis and treatment may result; however, the highly accurate VIDAS IgM test also gave negative results in these two samples; in follow-up sera obtained 2–3 weeks later, both the Elecsys and VIDAS IgM tests gave positive results.

Persistence of anti-*T. gondii* IgM antibodies has been described by us [16, 17] and others [18, 19]. Using immunosorbent agglutination and immunofluorescence tests, persistence of IgM antibodies has been observed for more than 2 years in up to 27% of pregnant women [20]. Interestingly, we observed that the Elecsys Toxo IgM test demonstrated shorter persistence compared to the VIDAS IgM test in sera from patients latently infected (>3 months) as well as in the seroconversion panels. This lower reactivity towards persistent Toxo IgM antibodies in samples from latently infected subjects has previously been reported [8] and may represent a clinical benefit since fewer sera (with positive IgM) need to be further investigated using additional tests or follow-up sera (=increased clinical specificity). Furthermore, unnecessary abortion has been shown to be prevented by appropriate use and interpretation of serological test results [21]. In newborns, we observed a relatively lower sensitivity of the Elecsys Toxo IgM test compared to ISAGA; this finding is confirming the superior sensitivity of ISAGA compared to immunoassays for the detection of IgM in newborns [22, 23]. In clinical prac-

tice, a battery of tests is commonly used to diagnose congenital infection including immunoassays for IgG, IgM, and IgA antibodies as well as ISAGA and PCR. The value of IgM immunoassays for the diagnosis of congenital infection should therefore be further investigated.

More recently, the Elecsys Toxo IgG avidity test was introduced to complete the diagnostic portfolio for *T. gondii*. A comparison of the Elecsys Toxo IgG avidity assay with two commercially available comparator assays revealed excellent performance characteristics to exclude recent infection [24]. Furthermore, the authors suggested investigating the value of very high and very low IgG avidity titers to exclude infections within the last 9 months or to confirm recent infections, respectively.

In conclusion, the present study revealed excellent reproducibility, no cross-reactivity, and excellent clinical accuracy of the Elecsys Toxo IgG and IgM tests. Combined with the rapid, fully automated high-throughput workflow solutions on the Cobas systems, the Elecsys Toxo IgG and IgM thereby provide an attractive laboratory offering to screen for and diagnose infection with *T. gondii*.

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