Evaluation of the New Elecsys Toxo IgG Avidity Assay for Toxoplasmosis and New Insights into the Interpretation of Avidity Results

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Detection and treatment of acute toxoplasmosis during pregnancy can avoid severe disease of the fetus. In this context, assessment of anti-Toxoplasma IgG avidity has been shown to exclude recent infection. The Elecsys Toxo IgG and IgM assays (Roche Diagnostics) have been validated for screening pregnant women and a new assay, Elecsys Toxo IgG Avidity, was recently developed. Our aims were to investigate the performance characteristics of this new avidity assay and explore whether additional information can be provided by avidity assays. The Elecsys assay was compared with the Vidas (bioMérieux) and Architect (Abbott) Avidity assays using two sets of serum samples (n = 291 and n = 255). The rate of general agreement between the Elecsys and Vidas assays was 74%, and that between the Elecsys and Architect assays was 83%. For 11% of the serum samples, avidity was high with the Vidas assay and within the gray zone with the Elecsys assay. None of the assays detected high-avidity antibodies in serum taken <4 months after infection. Avidity values of >90% were exclusively reported in sera taken >9 months after infection by the Elecsys and Architect assays. Almost all avidities of <19% with the Elecsys assay and <17% with the Architect assay corresponded to sera taken <3 and <2 months after infection, respectively. The Elecsys IgG Avidity assay can be used to exclude recent infection. New ways of interpreting the avidity result are also suggested: very high or low values could exclude infections within the last 9 months or help to confirm a recent infection, respectively. However, these potential interpretations require further investigation.

Primary infection by the apicomplexan parasite Toxoplasma gondii during pregnancy can result in severe disease of the fetus, such as neurologic or ocular lesions (15, 23, 24). As infection by T. gondii is generally asymptomatic, the diagnosis of infection in pregnant women relies on serologic assays. These tests are mandatory or highly recommended in several European countries (13, 21) and are also performed worldwide at the discretion of the physician. Suspicion or detection of toxoplasmosis acquired during pregnancy can be followed by measures intended to prevent fetal infection, such as maternal treatment with spiramycin or pyrimethamine-sulfadiazine, and fetal infection can be detected using fetal ultrasound and/or amniotic fluid PCR (15). More severe cases of congenital toxoplasmosis have been observed in countries where a standardized approach to diagnosis and treatment is not generally applied (13, 15, 16).

If a seronegative pregnant woman receives systematic monthly follow-up, assessment of T. gondii-specific immunoglobulin G (IgG) and IgM antibodies allows the date of seroconversion (and, hence, infection) to be estimated. The interpretation is, however, more difficult when a single sample is submitted for testing, for instance, when IgG and IgM are both found in the first serum sample submitted for testing during the pregnancy. These results may reflect a recently acquired infection—and, thus, a risk of transmission of the parasite to the fetus—or an infection acquired before the pregnancy, as T. gondii IgM antibodies may be detected for an extended period after seroconversion (4, 12, 18). In this situation, measurement of IgG antibody avidity has been shown to be useful; notably, a high IgG avidity excludes the possibility that the T. gondii infection occurred within the last 4 months (3 months for some assays) (1, 3, 6, 8, 14, 19, 22). A high avidity value can, therefore, allow the physician to avoid (or stop) unnecessary treatment, reassure the patient, and in some cases provide evidence that excludes the need to consider termination of pregnancy.

The Elecsys Toxo IgG and IgM assays (Roche Diagnostics GmbH, Mannheim, Germany) have been validated for screening and monitoring of immune status in pregnant women (18, 20). Recently, a new additional assay, the Elecsys Toxo IgG Avidity assay, has been developed. This assay is based on the Toxo IgG assay and is an in vitro diagnostic assay for the qualitative determination of the avidity of IgG antibodies against T. gondii in human serum and plasma. The aim of this study was to assess the reliability of the Elecsys Toxo IgG Avidity assay for estimating the time of onset of T. gondii infection in samples from pregnant women at various stages of infection and compare the results with those obtained using two other commercially available Toxo IgG Avidity assays.

MATERIALS AND METHODS

Study design. This study was conducted in the parasitology-mycology laboratories of two different teaching hospitals. The Elecsys Toxo IgG
Avidity assay (Roche) was compared with the Vidas Toxo IgG Avidity assay (bioMérieux, Marcy l’Étoile, France) at the laboratory in Marseille, France, and with the Architect Toxo IgG Avidity assay (Abbott Laboratories, Wiesbaden, Germany) at the laboratory in Grenoble, France.

Patients and sera. Each site provided its own set of serum samples collected from routinely screened pregnant women as well as from pregnant women at various stages of infection. All the samples included in the study were taken from women with a past or active seroconversion. A total of 546 samples were provided, 291 in Marseille and 255 in Grenoble. Each center classified the samples as follows: group A, samples collected from pregnant women with a recent primary infection, i.e., collected within 4 months of infection onset (n = 111 in Marseille, n = 94 in Grenoble); group B, samples collected from pregnant women 4 to 9 months after infection onset who did not have detectable anti- *T. gondii* IgM (n = 11 in Marseille, n = 7 in Grenoble); group C, samples collected from pregnant women 4 to 9 months after infection onset with detectable anti- *T. gondii* IgM (n = 94 in Marseille, n = 57 in Grenoble); and group D, samples collected from pregnant women more than 9 months after a primary infection (n = 75 in Marseille, n = 97 in Grenoble). In group D, samples were collected at the time of delivery from women with a periconceptional infection.

![FIG 1 Elecsys Toxo IgG Avidity assay principle. Samples are diluted in parallel with either the reference diluent (DilUni) or avidity diluent (DilToxoAv) and incubated for 10 min. During this time, IgG antibodies against *T. gondii* are bound to unlabeled *T. gondii*-specific recombinant antigen present in the avidity diluent. The remainder of the assay is fully automated and follows the one-step double-antigen sandwich principle. Briefly, the samples are incubated with a biotinylated recombinant *T. gondii*-specific antigen (surface antigen 1 [SAG1]) and a ruthenium-labeled *T. gondii*-specific antigen (surface antigen 1) to form a sandwich complex. In the sample preincubated with the avidity diluents, the labeled antigens compete with the unlabeled antigens already bound to IgG antibodies. As high-avidity antibodies bind more strongly to the antigen than low-avidity antibodies, competition with the unlabeled form of the antigen is more efficient; thus, there is less binding of labeled antigens in samples with high-avidity antibodies. Streptavidin-coated microparticles are then added and the complex binds to the solid phase via biotin-streptavidin interactions. The reaction mixture is transferred to a measuring cell and the microparticles are magnetically captured onto the surface of an electrode. Unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode, and chemiluminescence is measured by a photomultiplier. Avidity (in percent) can be calculated for samples with a reference measurement (i.e., an aliquot treated with reference diluent) of greater than 3 IU/ml according to the following equation: 100 \[ \frac{\text{concentration of aliquot treated with DilToxoAv}}{\text{concentration of aliquot treated with DilUni}} \times 100 \], where concentrations are in IU/ml.
TABLE 1 Cutoff values recommended by the manufacturer for each of the three avidity assays compared in this study

| Avidity assay          | Cutoff value or index for the following assay interpretation: |
|------------------------|-------------------------------------------------------------|
| Toxo IgG avidity assay | Low avidity | Gray zone | High avidity | |
| Elecsys (Roche Diagnostics) | <70%   | 70% ≤ value <80% | ≥80% | |
| Architect (Abbott Laboratories) | <50.0% | 50.0% ≤ value <60.0% | ≥60.0% | |
| Vidas (bioMérieux)     | <0.200 | 0.200 ≤ index <0.300 | ≥0.300 | |

* For all three assays, a high avidity value allows exclusion of a primary infection within the previous 4 months.

serocconversion or during pregnancy if the woman presented with stable anti-T. gondii IgG and undetectable IgM.

Evidence and the time of T. gondii infection had been previously established by testing the serum samples using routine techniques for the detection of anti-T. gondii IgG and IgM antibodies. In Grenoble, the samples had been tested using the following: Vidas Toxo IgGII and IgM assays (bioMérieux, Marcy l’Etoile, France), homemade IgG and IgM indirect immunofluorescence assays (2), and an IgM immunosorbent agglutination assay (Toxo ISAGA IgM; bioMérieux). In Marseille, they had been tested with Vida Toxo IgGII and IgM assays (bioMérieux), Toxo Screen DA assay (bioMérieux) and/or the LD-Bio Toxo II IgG immunoblot (LD-Bio, Lyon, France) and Toxo ISAGA IgM assay (bioMérieux).

All women from groups A, B, and C, as well as those from group D diagnosed with a periconceptional infection, were treated with either spiramycin or pyrimethamine-sulfonamide.

**Serologic assays.** In the present study, the Vidas Toxo IgGII (bioMérieux) and the Architect Toxo IgG (Abbott Laboratories) serologic assays were performed retrospectively on the 291 samples collected in Marseille and 255 samples collected in Grenoble, respectively. Every sample was tested with the Elecsys Toxo IgG Avidity assay using a Cobas e411 analyzer (Roche Diagnostics).

**Avidity measurements.** In accordance with the technical specifications, the Elecsys Toxo IgG Avidity assay was performed on all sera with an IgG titer greater than or equal to 6 IU/ml using the Elecsys Toxo IgG Avidity assay. The Elecsys Toxo IgG Avidity assay is a one-step double-antigen sandwich assay (Fig. 1).

All sera from Marseille with an IgG titer greater than or equal to 8 IU/ml using the Vidas Toxo IgGII assay were tested with the Vidas Toxo IgG Avidity assay using a Vidas analyzer (bioMérieux). This assay is fully automated and uses a two-step enzyme immunoassay sandwich method with a final fluorescent detection (14).

All sera from Grenoble with an IgG titer greater than or equal to 3 IU/ml using the Architect Toxo IgG assay were tested with the Architect Toxo IgG Avidity assay using an Architect analyzer (Abbott Laboratories). This assay is a fully automated chemiluminescent microparticle immunoassay (19).

Each avidity assay was used according to the manufacturer’s recommendations, and the manufacturer’s cutoff was applied to determine the reactivity of the sample (see interpretations in Table 1).

**Data analysis.** McNemar’s exact test for matched pairs was used to assess differences in the proportions of assasable sera and differences in the proportions of samples taken more than 4 months after infection that gave high avidity values. Cohen’s kappa value was calculated to evaluate the agreement between two assays; agreement with kappa values of 0.00 to 0.20 was considered slight, agreement with kappa values of 0.21 to 0.40 was considered fair, agreement with kappa values of 0.41 to 0.60 was considered moderate, agreement with kappa values of 0.61 to 0.80 was considered substantial, and agreement with kappa values of 0.81 to 1.00 was considered almost perfect (11). Pearson’s correlation coefficient was used to assess the statistical agreement between avidity values provided by the different assays. An avidity result was defined as discrepant if it was high with one assay and low with the comparator; a partially discrepant result was defined as being in the gray zone with one assay and low or high with the comparator.

**RESULTS**

**Proportion of assessable sera.** Some of the samples could not be assessed for IgG avidity due to a low IgG titer. The proportion of samples that could be tested for IgG avidity was dependent on the assay used, but for most sample groups, the proportions tested were similar between assays. However, the proportion of samples that could be tested using the Elecsys assay was statistically lower than that for the comparator assay for the following: overall data set from Marseille (94.3% for Elecsys versus 96.4% for Vidas; P = 0.031), group A samples from Marseille (84.8% for Elecsys versus 90.5% for Vidas; P = 0.031), and group A samples from Grenoble (72.3% for Elecsys versus 79.8% for Architect; P = 0.039).

**Correlations and general agreement between assays.** The calculation of Pearson’s coefficient showed a good correlation between the Elecsys and Architect assays (r = 0.77, n = 217) and Elecsys and Vidas assays (r = 0.73, n = 267) (Fig. 2). The general agreement of the Elecsys avidity assay with the Vidas assay was 74% (197/267 samples; kappa = 0.535, P < 0.01), and that with the Architect assay was 83% (180/217 samples; kappa = 0.646, P < 0.01). Out of 267 serum samples that could be analyzed with both the Elecsys and Vidas assays, 11 (4.1%) yielded discrepant results (Table 2); of the 217 serum samples analyzed with both the Elecsys and Architect assays, 8 (3.7%) yielded discrepant results (Table 3). All of these discrepant results corresponded to sera from women infected more than 4 months before sampling. Fifty-nine serum samples (22.1%; n = 267) analyzed with both the Elecsys and Vidas assays, as well as 29 serum samples (13.4%; n = 217) analyzed with the Elecsys and Architect assays, yielded partially discrepant results (Tables 2 and 3).

Between 7.8% and 19.5% of assessable serum samples yielded gray-zone avidity values, depending on the assay and serum group. In Marseille, this number was higher with the Elecsys than with the Vidas assay (n = 52 and n = 23 out of 267 samples, respectively), while in Grenoble it was similar between the Elecsys and Architect assays (n = 20 and n = 17 out of 217 samples, respectively). Notably, 30 serum samples (11.2% of all serum samples) were in the gray zone with the Elecsys assay, while they had high avidities with the Vidas assay (Table 2).

**IgG avidity results and time of infection.** As shown in Fig. 3, avidity values increased with time since infection for all three assays. However, these values were very scattered for both recent and past infections. The Elecsys, Vidas, and Architect assays gave high avidity values for 109 serum samples (32.5% of serum samples drawn more than 4 months after infection), 86 serum samples (48.3%), and 57 serum samples (36.7%), respectively. Test-to-test comparisons using McNemar’s test showed that the way that these sera were classified was significantly different between the Elecsys and Vidas assays (P < 0.001, n = 197) but not between the Elecsys and Architect assays (P = 0.228, n = 150). All the serum samples with a high avidity value had been drawn more than 4 months after infection onset. Hence, all three assays were able to exclude a recent primary infection (infection within the last 4 months) in samples with high-avidity antibodies. All the samples with an avidity value higher than 90% with the Elecsys (n = 28; 5.6% of the total number of serum samples, 16.6% of the >9-month serum samples) and Architect (n = 6; 2.6% of the total samples)
A number of serum samples, 6.5% of the >9-month serum samples) assays corresponded to sera collected more than 9 months after infection onset. This cutoff could be adjusted to 65% with the Architect assay (n = 52; 22.6% of the total number of serum samples, 56% of the >9-month serum samples), but with one exception: 1 serum sample with a high avidity value (87%) had been drawn 4 to 5 months after infection. Similarly, all the serum samples with an avidity value greater than 0.6 with the Vidas assay had been drawn more than 9 months after infection (n = 13; 4.8% of the total number of serum samples, 17.6% of the >9-month serum samples).

In all but one sample, avidity values less than 19% with the Elecsys assay corresponded to sera collected less than 3 months after infection onset (n = 32; 24% of the <3-month serum samples); the exception was a sample with a very low avidity value (7%) taken 5 to 6 months after infection onset. Similarly, all but one of the samples with an avidity of less than 17% with the Architect assay corresponded to sera collected less than 2 months after infection onset (n = 18; 35% of the <2-month serum samples). In this case, the exception was a sample with a low avidity value (13%) taken more than 9 months after infection.

**DISCUSSION**

The aim of this study was to evaluate the performance of the new Elecsys Toxo IgG Avidity assay on sera with well-defined dates of infection. Our findings indicate that the Elecsys Toxo IgG Avidity assay could be an interesting tool when used to exclude an infection that occurred in the previous 4 months. Indeed, all the sera with a high avidity were collected from patients at least 4 months...
after the onset of infection. Our findings and previously published studies show that this ability to exclude a recent infection, which is the ultimate goal of an avidity assay, is also shared by the Vidas and Architect Toxo IgG Avidity assays (8, 9, 17). In this study, the Vidas avidity assay appeared to be the most efficient assay because it was able to exclude a recent infection in nearly half of the samples taken more than 4 months after infection, whereas the Elecsys and Architect avidity assays were able to exclude a recent infection in one-third of the samples. According to previous studies, the ability to exclude recent infections was highly variable, ranging from 23% (8), 37.8% (7), and 90% (14) with the Vidas assay up to 72% with the Architect assay (9). The discrepancies between the results of these studies may be due to the proportion of sera from patients with an old infection in the tested samples. Indeed, our study shows that the group D sera, which correspond to the oldest infections, provided the highest proportions of high avidity values, as shown in Fig. 3.

Our comparison of the Elecsys avidity values with those of the competitor assays (Vidas and Architect) demonstrated good correlation coefficients. However, more informative are the assessments of agreement between Elecsys and each of the other two assays. When comparing the Elecsys and Architect assays, we found substantial agreement and low rates of discrepant or partially discrepant results, with very few samples showing high avidity with the Architect assay and gray-zone values with the Elecsys assay. The partial discrepancies between low and gray-zone values are not critical because they lead to the same conclusion; i.e., the possibility of recent infection cannot be excluded. A different set of samples was used to compare the Elecsys and Vidas assays, and more values were in the high-avidity zone with the Vidas assay than with the Elecsys assay, reflecting the moderate agreement between the two tests. This is particularly remarkable for recent infections, where no gray-zone values were observed with the Vidas assay, unlike with the comparator assays (Fig. 3).

We found a strong trend between very high avidity values ob-

| TABLE 2 Comparison of results obtained with Elecsys and Vidas assays on samples collected in Marseille |
|----------------------------------------------------|
| Elecsys IgG Avidity assay result | No. of samples with the following Vidas IgG avidity: |
|---------------------------------|----------------------------------|
|                                 | Low    | Gray zone | High | Total |
| Low                             | 139    | 14        | 6    | 159   |
| Gray zone                       | 14     | 8         | 30   | 52    |
| High                            | 5      | 1         | 50   | 56    |
| Total                           | 158    | 23        | 86   | 267   |

| TABLE 3 Comparison of results obtained with Elecsys and Architect assays on samples collected in Grenoble |
|----------------------------------------------------|
| Elecsys IgG Avidity assay result | No. of samples with the following Architect avidity: |
|---------------------------------|----------------------------------|
|                                 | Low    | Gray zone | High | Total |
| Low                             | 129    | 13        | 5    | 147   |
| Gray zone                       | 13     | 4         | 3    | 20    |
| High                            | 3      | 0         | 47   | 50    |
| Total                           | 145    | 17        | 55   | 217   |

FIG 3 Avidity values according to time since infection determined by the Elecsys (total number of serum samples \(n = 497\)) (A), Architect \((n = 230)\) (B), and Vidas \((n = 273)\) (C) assays. Each serum sample is represented by a dot. Dotted line, low-avidity and gray-zone cutoff; solid line, gray-zone and high-avidity cutoff. For each category (low avidity, gray zone, high avidity), the number of serum samples from each group is given within boxes. For each time point after infection, the median value is represented by a wide horizontal bar, while the median \(\pm 1\) standard deviation is represented by narrow horizontal bars.
tained using the three assays and sera collected more than 9 months after infection onset. We postulate that an additional assay threshold (e.g., 90% for the Elecsys and Architect assays and 0.65 for the Vidas assay) could be used to exclude infections in the previous 9 months, although this hypothesis would need further confirmation in extended studies. For diagnostic laboratories, this additional indication would be helpful in cases where the first \(T. gondii\) serologic assay is performed at delivery or between 4 and 9 months of pregnancy, by allowing exclusion of an infection during pregnancy. Furthermore, results of this study suggest that very low avidity values obtained with the Elecsys (<19%) or Architect (<17%) assays are associated with recent infections (onset less than 3 months and 2 months, respectively). Hence, the ability to assess avidity in these situations seems to be an important point, and we found that the samples from patients with very early infection (group A) were less frequently assessable with the Elecsys assay than with the comparator assays. Indeed, at the beginning of seroconversion, the Elecsys IgG titer is frequently equivocal and less than 6 IU/ml (the cutoff recommended by the manufacturer for performing the Elecsys avidity assay). However, it is remarkable that, at present, no commercial kit has been validated for confirming recent infection, although the ability of assays to report low avidity values for specimens drawn less than 4 months after seroconversion onset has already been reported in terms of sensitivity (10). Candolfi et al. also found that a low avidity value could firm up the diagnosis of an acute infection (5), but only samples taken very early (before treatment) or late (more than 1 year) in the disease course were used in the latter study (the lack of sera drawn 4 to 9 months after infection limits the chance of falsely detecting recent infections). More data are clearly needed to determine whether these avidity assays could be used to detect very recent infections and, if confirmed, to validate new cutoff values.

In conclusion, the Elecsys Toxo IgG Avidity assay was shown to be a valuable tool for excluding recent \(T. gondii\) infection. In addition, results from our evaluations led us to propose new ways of using avidity assay results: with the choice of appropriate thresholds, the Elecsys assay, as well as the two comparator assays used in our study, could be used to exclude an infection in the last 9 months when avidity values are very high; the Elecsys assay, as well as the Architect assay, could also be used to confirm a very recent infection when values are very low. These new findings, which should be confirmed in a larger panel of sera, may pave the way for additional uses for IgG avidity assays and help to resolve more clinical situations than we are able to today.

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