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Value of the IgG avidity in the diagnosis of recent toxoplasmosis: A comparative study of four commercially available anti-*Toxoplasma gondii* IgG avidity assays

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Abstract

The determination of the anti-*Toxoplasma* immunoglobulin G (IgG) avidity is a diagnostic method which has been intensively used for differentiating of recent and more distant *Toxoplasma gondii* infection in a single serum sample. Finding of low avidity anti-*Toxoplasma* IgG antibodies usually indicates recently acquired infection, while the presence of high avidity IgG antibodies contradicts to recently acquired infection. Recently, a number of commercial kits have been introduced for determining the avidity of anti-*Toxoplasma* IgG antibodies. Therefore, it seemed advisable to make comparative studies in order to investigate the correspondences and discrepancies in the results obtained by the different kits. We compared and analysed the results obtained with four commercially available anti-*Toxoplasma* IgG avidity assays. They were expected to give well correlated results, but some differences between the kits were observed in the ranking of anti-*Toxoplasma* IgG avidity ("high" or "low" or "borderline"). Among others, differences in the dilution method or differences in the type, quality or quantity of the *Toxoplasma* antigen used in the different assays might explain the observed divergences.

Key words

Toxoplasma gondii, congenital toxoplasmosis, serology, IgG avidity

Introduction

The acute (usually not the chronic) infection of women with *Toxoplasma gondii* during pregnancy may lead to severe sequels in the foetus (Remington and Klein 1995, Szénási *et al*. 1997, Liesenfeld *et al*. 2001). Since clinical symptoms are usually absent or non-specific, the *Toxoplasma* infection of the mother is detected in most cases by the demonstration of specific anti-*Toxoplasma* antibodies (Del Beno *et al*. 1989, Bobic *et al*. 1991, Liesenfeld *et al*. 2001).

The appearance of specific IgA, IgM, then IgG antibodies generally indicate the onset of an acute *Toxoplasma* infection. Few months later, the *Toxoplasma* specific IgA and IgM will diminish and/or disappear, while the specific IgG remains detectable till the end of the life and indicate the chronic stage of infection. In some patients, however, IgA and/or IgM anti-*Toxoplasma* antibodies may persist not just for some months but even years after acute infection. Therefore, in these cases, the presence of IgA and/or IgM antibodies will not indicate recently acquired *Toxoplasma* infection (Szénási *et al*. 1996, 1997; Liesenfeld *et al*. 2001).

In order to make reliable decision about anti-*Toxoplasma* chemotherapy, however, we should know, whether the infection is in the acute or chronic stage. Thus, we need supplementary methods, which can differentiate between the early and late stage of toxoplasmosis. Determination of the anti-*Toxoplasma* IgG avidity offers a possibility for such a differentiation.

The term "affinity" is only applicable to uniform and immunologically monovalent reactants and therefore, is confined to particular *in vitro* conditions (Hedman *et al*. 1993). In contrast, the terms "avidity" or "functional affinity" define the net antigen binding force of the populations of antibodies, and are preferable over the term "affinity" (Hedman *et al*. 1993).

Functional affinity of specific IgG antibodies, i.e. IgG avidity, initially is low after primary antigenic challenge, but increases during subsequent weeks and months by antigen driven B cell selection (Gergely 1998, Liesenfeld *et al*. 2001).

Toxoplasma IgG avidity can be measured by determining the antigen binding force of *Toxoplasma* specific IgG antibodies in a quantitative enzyme-linked immunosorbent assay

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(ELISA) (Hedman *et al*. 1989). In this assay, a hydrogen bond disrupting agent, urea, is used to elute IgG from the immobilised antigen. As a result, IgG antibodies of low avidity are almost completely dissociated in conditions where high avidity antibodies mostly remain antigen-bound (Hedman *et al*. 1989, Liesenfeld *et al*. 2001). The avidity is determined by using the ratios of antibody titration curves of urea-treated and -untreated samples (Liesenfeld *et al*. 2001). The presence of high avidity IgG antibodies can be used to rule out recently acquired infection (Montoya *et al*. 2002). Finding of low avidity antibodies usually indicates recently acquired infection. However, the low avidity antibodies can persist for many months after the acute infection in immunocompromised patients and in patients with anti-*Toxoplasma gondii* chemotherapy. In these cases, we cannot use the low avidity result for the diagnosis of recently acquired infection (Cozon *et al*. 1998, Pelloux *et al*. 1998, Liesenfeld *et al*. 2001, Montoya *et al*. 2002).

The IgG avidity test is a method that can differentiate between recent and more distant infection in a single serum sample, in the majority of cases (Liesenfeld *et al*. 2001). This test should assist in the diagnosis of acquired toxoplasmosis and may be used to identify pregnancies that are at risk for congenital toxoplasmosis (Hedman *et al*. 1989).

At present, a number of tests for the avidity of *Toxoplasma* IgG antibodies have been introduced to help the differentiation between recently acquired and distant infections (Hedman *et al*. 1989, Lappalainen *et al*. 1993, Holliman *et al*. 1994, Sensini *et al*. 1996, Gutiérrez *et al*. 1997, Jenum *et al*. 1997, Cozon *et al*. 1998, Pelloux *et al*. 1998, Liesenfeld *et al*. 2001, Montoya *et al*. 2002). The aim of our study was to compare four commercially available avidity assays and analyse the results in order to solve the divergences in the avidity results obtained by the different anti-*Toxoplasma* IgG antibody avidity tests.

Materials and methods

One hundred and two serum samples obtained from 56 patients were selected from a group of sera received in our laboratory for *Toxoplasma* serology determination, containing sera of patients suspected for fresh or past infection on the evidence of serological and clinical data. Ninety-six of the 102 sera were found to be anti-*Toxoplasma* IgG and IgM and/or IgA positive, and six of the 102 sera were anti-*Toxoplasma* IgG positive and IgM, IgA negative. Anti-*Toxoplasma* IgG antibody examination was performed by the bioMérieux "VIDAS Toxo IgG" enzyme-linked fluorescent assay (ELFA) kit (France), anti-*Toxoplasma* IgM and IgA determinations were performed by the Bio-Rad "PLATELIA Toxo IgM TMB" and the Bio-Rad "PLATELIA Toxo IgA TMB" ELISA (France), respectively. The anti-*Toxoplasma* IgG, IgM and IgA ELISAs were performed as described by the producers.

Toxoplasma IgG avidity tests were performed in all the 102 sera with (a) the bioMérieux "VIDAS Toxo IgG Avidity" ELFA kit (France) (hereafter abbreviated as VIDAS); (b) the TEST-LINE "EIA *Toxoplasma* IgG" ELISA (Czech Republic) (hereafter abbreviated as TESTLINE); (c) the Bio-Rad "PLATELIA Toxo IgG Avidity" ELISA (France) (hereafter abbreviated as PLATELIA); and (d) the DIESSE "Enzywell *Toxoplasma* IgG avidity" ELISA (Italy) (hereafter abbreviated as DIESSE). We used these tests according to the instructions given in their package inserts.

The VIDAS and the TESTLINE kits prescribe an anti-*Toxoplasma* IgG antibody concentration dependent dilution system in which first we determine the actual *Toxoplasma* specific IgG level of the examined sera. Then we dilute the sera so, that they should contain 15 (VIDAS) and 50 (TESTLINE) IU/ml of the *Toxoplasma* specific IgG, respectively.

The DIESSE kit requires a 1:100 dilution to be prepared independently of the actual *Toxoplasma* specific IgG level of the examined sera. The PLATELIA kit requires a 1:1000 dilution of sera with less than or equal to 240 IU/ml of specific anti-*Toxoplasma* IgG antibodies, but a 1:5000 dilution in other cases.

The avidity indexes were analysed and compared by correlation analysis and receiver-operator characteristic curve analysis (ROC-analysis) (GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego, California, USA). For expression of similarities and divergences in the results obtained by the different kits we used the following terminology [in agreement with the definition of Alvarado-Esquivel *et al*. (2002)]:

(1) Complete discrepancies: (a) high avidity in test kit 1 and low avidity in test kit 2, or (b) low avidity in test kit 1 and high avidity in test kit 2.

(2) Partial discrepancies: (a) high or low avidity in test kit 1 and borderline avidity in test kit 2, or (b) borderline avidity in test kit 1 and high or low avidity in test kit 2.

(3) Agreement: high, borderline, or low avidity in both 1 and 2 test kits.

Results

Table I shows the interpretation of the anti-*Toxoplasma* IgG avidity indexes as determined by the different kits and the number of sera classified as having low, borderline or high avidity. (Note that somewhat different cut-off levels and different intervals for exclusion of recent infection have been proposed by the different manufacturers.)

VIDAS: Twenty-nine sera (including 5 IgM and IgA negative sera) showed high avidity; avidity index (AI) was equal to or higher than 0.30, which according to the package insert excludes recent infection of <4 months; 10 sera (including 1 IgM and IgA negative serum) showed borderline avidity (AI was between 0.20 and 0.29) and 63 sera showed low avidity (AI was lower than 0.20). There were 22 sera collected from patients undoubtedly infected by *T. gondii* for more than 4 months (the serum sample was collected after the first anti-*T. gondii* IgG positive serum sample by 4 months). Testing these sera the VIDAS showed low avidity in 7 cases.

Table I. Interpretation of the avidity indexes of the different kits and the number of sera classified as having low, borderline or high avidity

Tests	Avidity						
	low		borderline		high		
	avidity index	number of sera	avidity index	number of sera	avidity index	number of sera	interpretation
VIDAS	0–0.19	63	0.20–0.29	10	0.30–1.00	29	infection was acquired >4 months ago
TESTLINE	0–0.30	34	0.31–0.35	5	0.36–1.00	63	infection was acquired >4 months ago
PLATELIA	0–0.39	54	0.40–0.49	11	0.50–1.00	37	infection was acquired >20 weeks ago
DIESSE	0–0.29	34	0.30–0.35	2	0.36–1.00	66	infection was acquired >3 months ago

TESTLINE: Sixty-three sera (including the 6 IgM and IgA negative sera) showed high avidity; AI was higher than 0.35, which according to the package insert excludes recent infection of <4 months; 5 sera showed borderline avidity (AI was between 0.31 and 0.35) and 34 sera showed low avidity (AI was equal to or lower than 0.30). There were 22 sera collected from patients undoubtedly infected by *T. gondii* for more than 4 months (the serum sample was collected after the first anti-*T. gondii* IgG positive serum sample by 4 months). Testing these sera the TESTLINE showed low avidity in 2 cases.

PLATELIA: Thirty-seven sera (including the 6 IgM and IgA negative sera) showed high avidity (AI was equal to or higher than 0.50). The package insert of PLATELIA determines <20 weeks for the exclusion of recent infection. Eleven sera showed borderline avidity (AI was between 0.40 and 0.49) and 54 sera showed low avidity (AI was lower than 0.40). There were 19 sera from patients undoubtedly infected by *T. gondii* for more than 20 weeks (the serum sample was collected after the first anti-*T. gondii* IgG positive serum sample by 20 weeks) and testing of these samples by PLATELIA kit resulted low avidity in 6 cases.

DIESSE: Sixty-six sera (including the 6 IgM and IgA negative sera) showed high avidity; AI was higher than 0.35, which according to the package insert excludes recent infection of <3 months; 2 sera showed borderline avidity (AI was between 0.30 and 0.35) and 34 sera showed low avidity (AI was lower than 0.30). Among the 102 serum samples there were 28 sera from patients undoubtedly infected by *T. gondii* for more than 3 months (the patients had have an anti-*Toxo-*

Table II. Correlation and interpretation of the avidity indexes of 4 different kits

	Correlation (r)	Agreements and discrepancies in the interpretation of the sera (number and %)		
	between the avidity indexes	agreement	partial discrepancies	complete discrepancies
VIDAS-PLATELIA	0.90 (p<0.0001)	84 (82.35)	13 (12.75)	5 (4.90)
VIDAS-TESTLINE	0.84 (p<0.0001)	64 (62.75)	13 (12.75)	25 (24.50)
VIDAS-DIESSE	0.69 (p<0.0001)	62 (60.78)	12 (11.77)	28 (27.45)
TESTLINE-DIESSE	0.81 (p<0.0001)	85 (83.33)	7 (6.86)	10 (9.81)
PLATELIA-TESTLINE	0.87 (p<0.0001)	71 (69.61)	14 (13.73)	17 (16.66)
PLATELIA-DIESSE	0.76 (p<0.0001)	68 (66.66)	13 (12.75)	21 (20.59)
VIDAS-TESTLINE (if borderline zone of TESTLINE is increased to 0.40–0.49)		69 (67.65)	21 (20.59)	12 (11.77)
VIDAS-TESTLINE (if borderline zone of TESTLINE is increased to 0.50–0.59)		80 (78.43)	14 (13.73)	8 (7.84)
VIDAS-DIESSE (if borderline zone of DIESSE is increased to 0.40–0.49)		64 (62.75)	16 (15.69)	22 (21.57)
VIDAS-DIESSE (if borderline zone of DIESSE is increased to 0.50–0.59)		66 (64.71)	16 (15.69)	20 (19.61)

plasma IgG positive serum sample at least 3 months before) and testing of these samples by the DIESSE kit resulted low avidity in 2 cases.

We calculated the correlation of the avidity indexes obtained with the 4 different kits and we examined the agreements or discrepancies of the interpretation in the cases of the 6 possible kit pair combinations (Table II).

The best correlation coefficient (r = 0.90) and the least complete discrepancies (4.90%) were found by comparing the VIDAS-PLATELIA kit pair. Those kits using a similar diluting method (VIDAS and TESTLINE) gave also a good correlation of 0.84, but a high proportion of complete discrepancies of 24.50%. The VIDAS-DIESSE kit pair gave the weakest correlation of avidity indexes (r = 0.69) and the most complete discrepancies (27.45%). The comparison of the TESTLINE and the DIESSE kits gave a "medium" correlation of 0.81 and a moderate degree (9.81%) of complete discrepancies. In the case of the kit pairs of PLATELIA-TESTLINE and PLATELIA-DIESSE the tendencies of correlation and similarities/discrepancies was parallel to the tendencies found in the case of the kit pairs VIDAS-TESTLINE and VIDAS-DIESSE (Table II).

The differences of the avidity results of sera obtained by the different kits might have been resulted to the differently defined cut-off values. This hypothesis is supported by the comparison of the VIDAS and the TESTLINE kit: in all cases of complete discrepancies (25 sera) as well as in all cases of partial discrepancies (13 sera) the TESTLINE kit gave higher avidity than the VIDAS. If we increase the borderline zone of the TESTLINE the number of partial and complete discrepancies considerably decreases (Table II). In the comparison of the VIDAS and the DIESSE kits in all cases of complete discrepancies (28 sera) and almost in all cases of partial discrepancies (11 of 12 sera) the DIESSE kit gave higher avidity than the VIDAS. If we increase the borderline zone of the DIESSE the number of partial and complete discrepancies fails to decrease considerably (Table II).

To verify the theory that the cut-off values proposed by the different manufacturers influence the interpretation of avidity a receiver-operator characteristic curve analysis (ROC-analysis) was performed. Three ROC-analyses were performed for each kit, where the "high avidity" interpretations of the results of the other 3 kits were used as standard in each analysis. According to the ROC-analysis the specificity of the TESTLINE kit using the current cut-off value (cut-off = 0.35) of high avidity is only 60.32% (95% CI: 47.20–72.43%) compared to VIDAS, 67.27% (95% CI: 53.29–79.32%) compared to PLATELIA and 88.24% (95% CI: 72.55–96.70%) compared to DIESSE. To reach an approximately 95% specificity the cut-off value of high avidity should be increased to 0.67 (compared to VIDAS), 0.65 (compared to PLATELIA) or 0.50 (compared to DIESSE). The ROC-analysis of VIDAS compared to the other three kits as controls suggests that the VIDAS kit is designed to be very safe concerning the specificity. The current cut-off value of high avidity (0.30) assures to the VIDAS kit a specificity of 100% compared to each other tests (TESTLINE 95% CI: 89.72–100%; PLATELIA 95% CI: 93.51–100%; DIESSE 95% CI: 89.72–100%). The ROC-analysis of PLATELIA kit compared to the other three kits as controls suggests a well defined cut-off value. The specificity of the PLATELIA kit using the current cut-off value (cut-off = 0.50) of high avidity is 93.65% (95% CI: 84.53–98.24%) compared to VIDAS, 100% (95% CI: 89.72–100%) compared to TESTLINE and 97.06% (95% CI: 84.67–99.93%) compared to DIESSE. The specificity of the DIESSE kit using the current cut-off value (cut-off = 0.36) of high avidity is similar to the specificity of the TESTLINE kit: 57.14% (95% CI: 44.05–69.54%) compared to VIDAS, 85.29% (95% CI: 68.94–95.05%) compared to TESTLINE and 63.64% (95% CI: 49.56–76.19%) compared to PLATELIA. To reach an approximately 95% specificity the cut-off value of high avidity should be increased to 0.86 (compared to VIDAS), 0.76 (compared to PLATELIA) or 0.64 (compared to TESTLINE).

Table III. Correlation between the avidity indexes of sera and the level of anti-*Toxoplasma* immunoglobulins

	VIDAS	TESTLINE	PLATELIA	DIESSE
IgG	r = 0.24 (p = 0.017)	r = 0.36 (p = 0.0002)	r = 0.36 (p = 0.0002)	r = 0.54 (p<0.0001)
IgM	r = –0.58 (p<0.0001)	r = –0.50 (p<0.0001)	r = –0.56 (p<0.0001)	r = –0.30 (p = 0.0022)
IgA	r = –0.32 (p = 0.011)	r = –0.23 (p = 0.0228)	r = –0.25 (p = 0.0107)	(not significant)

We performed a correlation analysis between anti-*Toxoplasma* IgG, IgM, IgA antibody levels and the avidity indexes of sera. We found a very weak positive correlation between the avidity indexes of the sera and the anti-*Toxoplasma* IgG antibody level of sera in the case of VIDAS (r = 0.24, p = 0.017), a weak positive correlation between the avidity indexes of the sera and the IgG level of sera in the case of TESTLINE (r = 0.36, p = 0.0002) and PLATELIA (r = 0.36, p = 0.0002), and a medium positive correlation between the avidity indexes of the sera and the anti-*Toxoplasma* IgG antibody level of sera in the case of DIESSE (r = 0.54, p<0.0001). In the case of VIDAS, TESTLINE and PLATELIA, we found a relatively good negative correlation between the avidity indexes and the anti-*Toxoplasma* IgM antibody level of sera (VIDAS: r = –0.58, p<0.0001; TESTLINE: r = –0.50, p<0.0001; PLATELIA: r = –0.56, p<0.0001) and a modest negative correlation between the avidity indexes and the anti-*Toxoplasma* IgA antibody level of sera (VIDAS: r = –0.32, p = 0.011; TESTLINE: r = –0.23, p = 0.0228; PLATELIA: r = –0.25, p = 0.0107). In the case of the DIESSE we found a weak negative correlation between the avidity indexes and the

anti-*Toxoplasma* IgM antibody level of sera ($r = –0.30$, $p = 0.0022$) and no correlation between the avidity indexes and the anti-*Toxoplasma* IgA antibody level of sera (not significant, $p>0.05$) (Table III).

Discussion

The *Toxoplasma* specific IgG avidity test is a method that can generally differentiate the recent and more distant infection in a single serum sample (Liesenfeld *et al*. 2001, Montoya *et al*. 2002). Recently, a number of tests for the avidity of *Toxoplasma* IgG antibodies have been introduced. The aim of our study was to compare four commercially available avidity assays and analyse the results in order to solve the divergences in the avidity results obtained by the different anti-*Toxoplasma* IgG antibody avidity tests. They were expected to give well correlating results, but this expectation was not confirmed by this study.

The best correlation coefficient of avidity indexes ($r = 0.90$, $p<0.0001$) and the second best agreement (82.35%) belong to the VIDAS-PLATELIA kit pair. VIDAS and TESTLINE use a diluting method, in which first we determine the actual *Toxoplasma* specific IgG level of the examined sera. Then we dilute the sera so, that they should contain 15 (VIDAS) and 50 (TESTLINE) IU/ml of the *Toxoplasma* specific IgG, respectively. In spite of the similar diluting method, the VIDAS and TESTLINE kit pair showed a weak agreement (62.75%), but they gave a good correlation ($r = 0.84$, $p<0.0001$). This is not a contradiction, because the results fitted to the regression line well, but the results of the TESTLINE were consequently much higher than the results of the VIDAS. The correlation between the avidity indexes of pairs of IgG concentration dependent kits (VIDAS, PLATELIA, TESTLINE) varied between $r = 0.84–0.90$. The weakest correlation belonged to the VIDAS-TESTLINE kit pair ($r = 0.84$). If we increase the borderline zone of the TESTLINE the number of partial and complete discrepancies considerably decreases (Table II). In the comparisons of IgG concentration dependent kits and the IgG concentration independent kit (DIESSE) this correlation was $r = 0.69–0.81$. The weakest correlation belonged to the VIDAS-DIESSE kit pair ($r = 0.69$). If we increase the borderline zone of the DIESSE the number of partial and complete discrepancies fails to decrease considerably (Table II). Therefore, in the comparison of the different *T. gondii* IgG avidity tests the dilution method of the sera seems to be more important influencing factor than the appropriate choice of the cut-off value in the number of sera showing agreement and partial or complete discrepancies.

The possibility arose, that through the dilution ratio, the *Toxoplasma* specific IgG level of the sera can influence the avidity indexes. Therefore, we looked for a correlation between the avidity indexes of the sera and the IgG level of the sera in the case of the four kits. By *Toxoplasma* specific IgG concentration dependent dilution of the sera, the VIDAS and the TESTLINE as well as PLATELIA try to eliminate the unfavorable effect of high *Toxoplasma* specific IgG concentration. As a result, we found a very weak positive correlation between the avidity indexes of the sera and the *Toxoplasma* specific IgG level of sera in the case of VIDAS, a weak positive correlation between the avidity indexes of the sera and the *Toxoplasma* specific IgG level of sera in the case of TESTLINE and PLATELIA, and a medium positive correlation between the avidity indexes of the sera and the *Toxoplasma* specific IgG level of sera in the case of DIESSE. In the case of VIDAS, TESTLINE and PLATELIA, we found a relatively good negative correlation between the avidity indexes and the *Toxoplasma* specific IgM level of sera, and a modest negative correlation between the avidity indexes and the *Toxoplasma* specific IgA level of sera. Maybe it can be attributed to the unfavorable effect of the high anti-*Toxoplasma* IgG antibody level that in the case of the DIESSE we found a poor negative correlation between the avidity indexes and the anti-*Toxoplasma* IgM antibody level of sera and no correlation between the avidity indexes and the anti-*Toxoplasma* IgA antibody level of sera in spite of the fact that the high IgM and IgA levels are the predictors of the acute infection as well as the low avidity (Table III).

Our examination showed that the dilution of the sera seems to be important in respect to the comparability of the results. A possible explanation might be that in those tests, which work with a single predetermined serum dilution, independently from the actual antibody level of the given sera does not contain the optimal amount of IgG in the given dilution, and thus, the amount of antigen-antibody complex does not fit completely to the amount of urea.

This fact shows that the diluting method is an important factor in respect to the similarity of avidity indexes and interpreted results, but it is not the only influencing factor. The ROC-analysis showed that the approach of the different kits is different. Some kits (e.g. VIDAS, PLATELIA) are designed to be very safe concerning the specificity. These kits are able to select the high avidity sera, the specificity of the VIDAS kit (compared to the interpretation results of the other three kits) is 100%. The advantage of this high specificity is that we can rule out the fresh infection in the case of high avidity.

Gutiérrez *et al*. (1997) compared two commercially available IgG avidity ELISA kits (Sorin ETI Toxo G, Sorin Biomedica, Italy and Enzygnost, Behring, Germany). They found a high correlation between results in both tests. Alvarado-Esquivel *et al*. (2002) compared other two commercially available IgG avidity ELISA kits (VIDAS Toxo IgG Avidity, bioMérieux, France and *Toxoplasma gondii* IgG Avidity, Labsystems, Finland). They found a good agreement of 75%. In contrast, in the study of Barberi *et al*. (2001) poor correlation of 37.2% was found among three commercially available *Toxoplasma* IgG avidity tests (Sorin, Labsystems, bioMérieux). In our study we compared four commercially available *Toxoplasma* IgG avidity tests, and correlations between the avidity indexes of the kit pairs were 0.69–0.90, the agreement was between 60.78 and 83.33%.

The main problem is that the above mentioned differences experienced in the kits give a chance for different conclusion in some cases. Finding of low avidity antibodies usually indicates recently acquired infection. The presence of high avidity IgG antibodies can be used to rule out recently acquired infection (Montoya *et al*. 2002).

The low avidity antibodies can persist for many months after the acute infection in immunocompromised patients and in patients with anti-*T. gondii* chemotherapy. In these cases, we cannot use the low avidity result for the diagnosis of recently acquired infection (Cozon *et al*. 1998, Pelloux *et al*. 1998, Liesenfeld *et al*. 2001, Montoya *et al*. 2002).

At the present time, data suggest that the avidity test represents a valuable additional confirmatory method, which is most useful if high avidity antibodies are detected (Liesenfeld *et al*. 2001, Montoya *et al*. 2002). It should not be used, however, as a single confirmatory test for pregnant women with IgG and/or IgM antibodies because of the potential for misinterpretation the low or borderline avidity results (Liesenfeld *et al*. 2001, Montoya *et al*. 2002). The high avidity result can only say that the infection has occurred four or more months before, but cannot say precisely the clinical stage of the infection.

Testing with the avidity method in pregnant women during the first 16 weeks of gestation has the potential to decrease the need for follow-up sera, and also the need of performing PCR on amniotic fluid. It decreases the chance of unnecessary treatment of the mother with spiramycin or other drugs and thereby reduces costs. Also, it decreases the chances of the unnecessary and unwanted abortions. In addition, it eliminates the pregnant woman's anxiety associated with further serum testing (Liesenfeld *et al*. 2001, Montoya *et al*. 2002). However, we have to admit, that there is no method or combination of methods, which can predict the exact time of the infection within the first year after infection (Petersen *et al*. 2001).

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