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## **Toxoplasma gondii-specific IgG avidity testing in pregnant women**

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1 **Abstract**

2

3 **Background**

4 The parasite *Toxoplasma gondii* (Tg) can cause congenital toxoplasmosis following primary infection  
5 in a pregnant woman. It is therefore important to distinguish between recent and past infection  
6 when both Tg-specific IgM and IgG are detected in a single serum in pregnant women. Tg-specific IgG  
7 avidity testing is an essential tool to help to date the infection. However, interpretation of its results  
8 can be complex.

9 **Objectives:**

10 To review Tg-specific avidity testing benefits and limitations in pregnant women, in order to help  
11 practitioners to interpret the results and adapt the patient management.

12 **Sources:**

13 PubMed search with the keywords avidity, toxoplasmosis and *Toxoplasma gondii* for articles  
14 published from 1989 to 2019.

15 **Content:**

16 Tg-specific IgG avidity testing remains a key tool for dating a Tg infection in immunocompetent  
17 pregnant women. Several commercial assays are available and display comparable performances. A  
18 high avidity result obtained on a first-trimester serum sample is indicative of a past infection, which  
19 occurred before pregnancy. To date, a low avidity result must still be considered as non-informative  
20 to date the infection, although some authors suggested that very low avidity results are highly  
21 suggestive of recent infections depending on the assay. Interpretation of low or grey zone avidity  
22 results on a first-trimester serum sample, as well as any avidity result on a second or third-trimester  
23 serum sample, is more complex and requires recourse to expert toxoplasmosis laboratories.

24 **Implications:**

25 Although used for about 30 years, Tg-specific avidity testing has scarcely evolved. The same  
26 difficulties in interpretation have persisted over the years. Some authors proposed additional

- 27 thresholds to exclude an infection of less than 9 months, or in contrast to confirm a recent infection.
- 28 Such thresholds would be of great interest to adapt management of pregnant women and avoid
- 29 unnecessary treatment: however, they need confirmation and further studies.

1 **Introduction**

2 *Toxoplasma gondii* (Tg) is a cosmopolitan intracellular parasite. Congenital toxoplasmosis can occur  
3 following primary infection in a pregnant woman. The risk of transplacental transmission and severity  
4 of foetal disease depend on the gestational age at the time of maternal infection [1,2]. As the  
5 infection is usually asymptomatic in pregnant women, toxoplasmosis detection and screening during  
6 pregnancy rely on serological techniques. While detection of both Tg-specific IgM and IgG in a single  
7 serum sample must suggest an acute infection, a past infection cannot be excluded either since Tg-  
8 specific IgM antibodies can persist for months or years after infection (Figure 1, Table 1). Tg-specific  
9 IgG-avidity testing can be helpful in discriminating between these two possibilities, but its  
10 interpretation can be complex and sometimes confusing for non-experts. This review is designed to  
11 support interpretation of complex Tg serological results, and focuses on the benefits and limitations  
12 of Tg-specific IgG-avidity testing in pregnant women. It is based on a literature research in Pubmed  
13 using the keywords avidity, toxoplasmosis and *Toxoplasma gondii* from 1989 to 2019.

14

15 **What is avidity?**

16 Avidity, or functional affinity, is a measure of the binding strength of IgG antibodies to an antigen.  
17 Avidity increases with time, as, following prolonged or repeated exposure to the antigen, the IgG  
18 hypervariable regions successively adapt through antigen-driven B cell selection, to bind to it more  
19 tightly [3]. Measuring the avidity of specific IgG-antibodies directed against a given pathogen,  
20 e.g. *Toxoplasma gondii*, can therefore help to discriminate between a recent and a past infection.

21 Avidity testing is of particular interest in helping to date a *T. gondii* (Tg) infection in pregnant women  
22 after both specific IgM and IgG have been detected, and guide the patient management (see below).

23

24 **How to measure avidity?**

25 Several approaches exist for the measurement of Tg-specific IgG avidity. Most of the techniques rely  
26 on the use of a protein denaturing agent, e.g. hypermolar urea (4-8M), as a diluent or a washing

27 agent (Table 1). This denaturing agent either prevents the formation (diluent) or allows the  
28 dissociation of antigen/low-avidity antibodies complex by disruption of hydrogen bonds (washing  
29 agent). Basically, Tg-specific IgG testing is performed twice in parallel, with and without denaturing  
30 agent, on a single dilution or serial dilutions of serum. Avidity is then calculated as the ratio of IgG  
31 testing (optical density, luminescence or IgG titre) with denaturing agent (corresponding to high-  
32 avidity specific IgG antibodies) to IgG testing without denaturing agent. The result is expressed as an  
33 index (Avidity Index (AI)) or a percentage. As the relationship between OD and IgG titre (UI/mL) is not  
34 linear, the result can vary according to the mode of calculation [4,5].

35 Other techniques, i.e. Architect or Alinity Toxo IgG avidity (Abbott Diagnostics) and Elecsys Toxo IgG  
36 Avidity (Roche Diagnostics) assays, use recombinant antigens as blocking agents. These soluble  
37 blocking agents bind to and neutralize high-avidity specific IgG antibodies. As the previous  
38 techniques, two reactions are performed in parallel, with or without blocking agent, on a single-  
39 dilution of serum, but by contrast, low-avidity specific IgG antibodies are detected. Avidity (%) is  
40 therefore calculated according to the following formula:  $[1 - (\text{IgG testing with blocking agent}/\text{IgG}$   
41  $\text{testing without blocking agent})] \times 100$ .

42  
43 Avidity testing can be performed either on serial dilutions (end-titre method) or on a single dilution  
44 of serum. The automated commercial assays, detailed in Table 1, measure avidity using the single  
45 dilution serum method. As AI can vary according to the total concentration of IgG in the sample [6],  
46 some manufacturers recommend adjusting each serum concentration within a standard range of IgG  
47 titres before measuring avidity. In addition, all the assays require a minimal IgG titre to be  
48 performed. The end-titre method is considered the gold-standard as it does not depend on IgG  
49 concentration, but it is not adapted for routine diagnosis [6,7].

50

## 51 **Interpretation of Tg-specific IgG avidity results**

52 The aim of avidity testing is to discriminate between recently-acquired and past infections. Briefly, a  
53 high Tg-specific IgG avidity result strongly indicates a past infection, while a low or intermediate  
54 avidity result is not informative in dating the infection. However, rules of interpretation vary  
55 according to the assay, notably the definition of time elapsed for infection to be considered acute or  
56 past, the degree of certainty in ruling out a recent infection with a high avidity result, and the  
57 interpretation of a low avidity result (Table 1). Most of the manufacturers consider a past infection as  
58 an infection older than 4 months, whereas some others set a time frame of more than 3 months  
59 (Chorus or Enzywell Toxoplasma IgG avidity, Dienes Diagnostica Senese) or 20 weeks (Platelia™ TOXO  
60 IgG Avidity, Biorad). Some assays interpret a high avidity result as ruling out a recent infection, while  
61 others do not exclude a recent infection with complete certainty. Similarly, some manufacturers  
62 interpret a low avidity result as suggesting a possible but not confirmed recent infection (Biorad,  
63 Diasorin, Vircell, Technogenetics), while others offer no interpretation of low avidity results  
64 (bioMerieux, Abbott, Roche).

65

66 Despite this general principle of interpretation and the specifications of the different manufacturers,  
67 many questions persist about avidity. One of them is: can a recent infection be ruled out reliably with  
68 a high avidity result? The answer is "yes". The ability of high avidity results to exclude a recent  
69 infection has been shown for all commercial assays currently available, with Positive Predictive  
70 Values (PPV) very close or equal to 100% [8–12]. According to some authors, a very high avidity result  
71 (>90%) with the Elecsys (Roche Diagnosis) and Architect (Abbott) assays could even rule out an  
72 infection of <9 months [8,12]. High avidity results have been only exceptionally reported in cases of  
73 recent infections and have not been explained to date [10,13].

74

75 Another frequent but complex question about avidity is: can a low avidity result confirm a recent  
76 infection? The answer is "no, but". To date, a low avidity result must still be considered as non-  
77 informative in dating the infection. Indeed, persistent low avidity results have been commonly

78 reported in serum from patients with past infections of months or even years, whatever the  
79 technique used. One study estimated the PPVs of a low avidity result at 61.1%, 73.5%, 74.3% and  
80 77.5% for the Platelia, Architect, Liaison and VIDAS assays, respectively, and a similar PPV was found  
81 for the Elecsys assay: 69.9% [8,11,12,14–16]. The reasons for delayed (or absence of) Tg-specific IgG  
82 maturation in some patients have not yet been fully elucidated.[17] Considerable inter-individuals  
83 variations have been observed [5,18]. Tg-specific IgG maturation is delayed in pregnant women and  
84 in neonates with congenital toxoplasmosis.[15,19,20] Although controversial, it has been suggested  
85 that anti-*Toxoplasma* treatment, e.g. with spiramycin, may lead to delayed Tg-specific IgG  
86 maturation in pregnant women by reducing the parasite load [5,15,16,20,21]. In a study in mice  
87 comparing different antiparasitic treatment regimens, only atovaquone was observed to impact  
88 avidity maturation, whereas spiramycin or pyrimethamine-sulfadiazine were not [22]. In addition,  
89 some authors did not find any difference in avidity between treated or untreated pregnant women  
90 [16]. Finally, the kinetics of IgG avidity increase over time varies between assays [8,11,23]. The  
91 frequency of persistent low avidity results in past infection is difficult to estimate accurately, as most  
92 of the studies on avidity are retrospective and performed on selected samples from bank sera.

93 While a low avidity result cannot confirm a recent infection, some authors suggested that a very low  
94 avidity result could be highly suggestive of this [8,12,24,25]. Among them, Fricker-Hidalgo *et al* and  
95 Murat *et al* concluded that Tg-specific IgG avidity results  $\leq 15\%$  with the Elecsys assay or  $< 17\%$  with  
96 the Architect assay could reliably confirm recent infections of  $< 3$  or  $< 2$  months, respectively [8,12].  
97 Similarly, Boquel *et al* reported that a very low avidity result  $< 0.05$  with the Liaison XL Toxo IgG avidity  
98 assay was indicative of a probable recent infection of  $< 3$  months, with a PPV of 97% [25]. To date,  
99 and until additional studies are performed, the hypothesis of a very recent infection based on a very  
100 low avidity result should be confirmed by repeat Tg-specific IgM and IgG testing on a serum sample  
101 taken 2-3 weeks later.

102



103 As stated above, there are some differences in interpretation of an avidity result according to the  
104 assay, but are the overall performances of the different commercial assays comparable? The answer  
105 appears to be "yes", since many studies comparing 2 to 4 Tg-specific avidity assays in parallel found  
106 that avidity values were highly correlated whatever the technique (correlation coefficients  $\geq 0.795$ ),  
107 with similar kinetics of avidity maturation [8,11,26–29]. General agreement between assays was  
108 good, around 80% [8,27,30]. Although the oldest (developed in 1998) and the cheapest, the VIDAS  
109 assay performed best for the diagnosis of past infections [11,27,31,32]. In a study, the accuracy  
110 compared to the date of infection was 93.4% vs 86.8% for the VIDAS and the Architect assays  
111 respectively [31]. Several studies also suggested that AI in the grey zone with the VIDAS assay could  
112 rule out a recent infection of <4 months [16,31]. A similar observation was made with the Architect  
113 assay [31]. This last assay was the most dynamic one in recent infections, probably due to the use of  
114 early and late recombinant antigens [11]. The Elecsys assay, also based on the use of recombinant  
115 antigens, appeared correlate well (83%) with the Architect assay [8]. Due to these inter-assay  
116 variations, the use of two different techniques for avidity testing could be helpful in some particular  
117 situations (see below and Figure 1) [30,33].

118

### 119 **Tg-specific IgG avidity testing in pregnant women**

120 Tg-specific IgG avidity testing is mainly performed in pregnant women. It is recommended in an  
121 immunocompetent pregnant woman displaying both IgM and IgG on first serological testing, in  
122 addition to follow-up serological testing 2-3 weeks later, in order to distinguish between acute and  
123 latent infection and therefore adapt the patient management.

124

125 Interpretation of high, grey-zone or low avidity results depends on the term of pregnancy at the time  
126 of sampling (Figure 1). First, what about high avidity results? In several European countries including  
127 France, some regions of Italy and Austria, a toxoplasmosis serological screening is recommended  
128 during the first trimester for any pregnant women [34,35]. Avidity testing is performed when both

129 Tg-specific IgG and IgM are detected in this screening sample. In this situation, interpretation of a  
130 high avidity result is straightforward, as the serum is taken early in pregnancy: it allows identification  
131 of a past infection, which occurred before the beginning of pregnancy, with residual IgM [24].  
132 Monthly serological screening can therefore be stopped (provided stable antibody titres are found in  
133 a second serum taken 3 weeks later). Even if rare, it must be kept in mind that foetal transmission of  
134 *T. gondii* can occur following anteconceptional infection (usually in the 2 months prior to  
135 conception): consequently, a high avidity result in a serum sampled at the end of the first trimester  
136 must be interpreted with caution [36]. Avidity testing can also be performed later in pregnancy in  
137 countries in which no systematic toxoplasmosis screening is implemented or in pregnant women  
138 with no previous follow-up. To date, a high avidity result obtained in a serum sampled >4 months  
139 after the beginning of pregnancy does not exclude an infection during pregnancy. Study of the Tg-  
140 specific IgG and IgM kinetics +/- additional techniques (e.g. Tg-specific IgA testing) are therefore  
141 required. Antiparasitic treatment, ultrasound follow-up as well as amniocentesis could also be  
142 offered to these pregnant women on a case-by-case basis in order to prevent and/or manage  
143 congenital toxoplasmosis [1]. In these situations, additional thresholds allowing the exclusion of  
144 infections <9 months would be of great value. As mentioned above, such thresholds have already  
145 been suggested, but for only two commercial assays and they need to be confirmed in further studies  
146 [8,12].

147 Interpretation of grey-zone avidity results during pregnancy is more complex. Currently, unless there  
148 is perfect stability of antibody titres in 2 sera taken 2-3 weeks apart, they are most often interpreted  
149 as non-informative in dating the infection, whatever the gestational term. As an infection during  
150 pregnancy therefore could not be firmly ruled out, antiparasitic treatment and prenatal diagnosis of  
151 congenital toxoplasmosis are implemented according to national or local guidelines. However, if  
152 obtained on a first-trimester sample, it may be helpful to investigate a grey-zone avidity result with a  
153 different avidity testing method, i.e the VIDAS assay which displayed the best performances for the  
154 diagnosis of past infections, which could possibly give a high avidity result instead [11,30,31]. Some

155 authors also suggested that grey-zone avidity results obtained with the Architect and VIDAS assays  
156 could exclude a recent infection of <4 months, but again, it needs confirmation [16,31].

157 As mentioned above, a low avidity result must be considered as non-informative for dating the  
158 infection, and must lead to the implementation of measures for prevention and diagnosis of  
159 congenital toxoplasmosis (see above) whatever the gestational term, without delay. These measures  
160 are especially required in case of a very low avidity result as it has been shown to be highly  
161 suggestive of a very recent infection, and/or in presence of both high Tg-specific IgM titres and IgG  
162 which could also indicate a recent infection [8,12,25].

163  
164 As a result, because of complex interpretation, and with the exception of a high avidity result in the  
165 very beginning of pregnancy, avidity testing in pregnant women must be referred to an expert  
166 toxoplasmosis laboratory.

167  
168 IgG maturation and host-parasite dynamics can be altered in immunocompromised patients, making  
169 interpretation of avidity results even more difficult [11]. Therefore, in that context, screening for  
170 toxoplasmosis infection during pregnancy should rather rely on monthly serological testing +/- PCR,  
171 whatever the serological status.

172  
173 **Alternatives to avidity testing /perspectives**

174 Despite its aforementioned limits, avidity testing remains an essential tool: to date, it is the most  
175 reliable diagnostic tool for the discrimination between acute and past toxoplasmosis in a single  
176 serum.

177  
178 Performing Tg-specific IgA testing in association with avidity testing in pregnant women could also  
179 help to distinguish between a recent and a chronic Tg infection. Indeed, in a recent study, Olariu *et al*  
180 showed that the prevalence of IgA antibodies decreased when AI increased. IgG+, IgM+ and IgA+

181 pregnant women were more likely to have had a recent infection than IgG+, IgM+ and IgA- ones [37].  
182 However, a recent infection could not be excluded on the absence of Tg-specific IgA alone, and these  
183 antibodies can persist for months after infection [37,38].

184  
185 Improvements in avidity testing would probably consist of the use of recombinant antigens or  
186 chimeric peptides [39,40]. Kinetics of IgG maturation differ against native versus recombinant  
187 antigens, as well as against one recombinant antigen and another [40]. Using this property could help  
188 to discriminate better between recent and past infections. To date, two commercial fully automated  
189 avidity testing assays are already based on recombinant antigens: the Architect/Alinity (SAG1, GRA8)  
190 and the Elecsys ones (SAG1) (Table 1) [29]. Another qualitative assay for the determination of Tg-  
191 specific IgG avidity, although less common, uses recombinant antigens: recomLine Toxoplasma IgG  
192 [Avidity] (Mikrogen). Other potentially interesting recombinant antigens, used alone or in  
193 association, have been identified including MIC3, GRA1, GRA6, GRA7, SAG2 and ROP1 [41–45].  
194 Recombinant chimeric peptides, composed of different epitopes selected from Tg antigens, have also  
195 been generated and tested for their potential in toxoplasmosis serological diagnosis [39,40,46].  
196 Among them, the recombinant multi-epitope peptide (rMEP) developed by Dai *et al* showed  
197 promising results for the discrimination of recent from late infections [46].

198

## 199 **Conclusion**

200 Tg-specific IgG avidity testing is an essential tool to help to date an infection when both IgM and IgG  
201 are detected in a single serum in pregnant women (Figure 1). Although used in diagnosis for about 30  
202 years, it has scarcely evolved. The same difficulties in its interpretation have persisted over the years,  
203 requiring recourse to expert toxoplasmosis laboratories. Definition of additional thresholds to  
204 exclude an infection of less than 9 months would be of particular value during pregnancy when both  
205 IgG and IgM are detected. In addition, confirmation of a recent *T. gondii* infection by a low avidity  
206 result would be useful in avoiding unnecessary treatment of pregnant women. To date, only a very

207 few studies have suggested such thresholds; confirmation of these results and additional assays are  
208 both required.

209

210

211 **Transparency declaration:**

212

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219

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Table 1: Automated commercial Tg-specific IgG avidity testing assays

	<b>bioMérieux Vidas® Toxo IgG Avidity</b>	<b>Biorad Platelia™ TOXO IgG Avidity</b>	<b>Diasorin Liaison® XL Toxo IgG Avidity</b>	<b>Vircell Toxoplasma Virclia® IgG Avidity monotest</b>	<b>Technogenetics Immunodiagnosti c Systems IDS-iSYS/TGS TA Toxo IgG Avidity</b>	<b>Abbott Architect®/Alinity® Toxo IgG Avidity</b>	<b>Roche Elecsys® Toxo IgG Avidity</b>
<b>Principle of reaction</b>	ELFA	Indirect EIA	CLIA	CLIA	Indirect CLIA	CMIA	CLIA
<b>Dissociation or blocking agent</b>	Urea	Urea	Urea	Urea	ND	Recombinant proteins	Recombinant proteins
<b>Antigens</b>	<i>Toxoplasma</i> Lysate Antigen	ND	<i>Toxoplasma</i> Lysate Antigen	ND	<i>Toxoplasma</i> Lysate Antigen	P30 (SAG1) P35 (GRA8)	P30 (SAG1)
<b>Automation</b>	Semi-automated	Manual and automated	Automated	Automated	Automated	Automated	Automated
<b>Requirements of the avidity reaction</b>	IgG ≥ 8 IU/mL IgG >15 IU/mL: to be diluted	IgG ≥ 9 IU/mL	IgG ≥ 8.8 IU/mL IgG <15 IU/mL: interpretation with caution	Antibody index ≥1.1	Protocol depending on the IgG titre (1,5-5;5<IgG<50 ;>50)	IgG ≥ 1.6 IU/mL	IgG ≥ 6 IU/mL IgG >500 IU/mL: to be diluted
<b>CE-IVD</b>	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<b>Interpretation</b>	Index Low avidity <0.20 Grey zone 0.20≤AI<0.3 High avidity ≥0.30	Index <0.40 0.40≤AI<0.5 ≥0.50	Index <0.20 0.20≤AI<0.3 ≥0.30	Index <0.4 0.4-0.5 >0.5	Index ≤0.1 0.1<AI≤0.15 >0.15	Percentage <50% 50-59.9% ≥60%	Percentage <70% 70-79% ≥80%
<b>Meaning of :</b>							
Low avidity	Not a proof of recent infection	Suggestion of a recent infection of <20 weeks	Suggestion of a primary infection acquired within the last 4 months	In favor of recent primo-infection of less than 4 months	Suggestion of a primary infection acquired within the last 4 months	Cannot be used to diagnose an acute infection	No clinical interpretation
High avidity	Strongly suggests an infection of >4 months	Suggestion of a past infection of >20 weeks	Exclusion of a primary infection acquired within the last 4 months	In favor of past-infection of more than 4 months	Exclusion of an infection acquired within the last 4 months	Strong indication of an infection of >4 months	Exclusion of an infection acquired within the last 4 months

<b>References</b>	[5,11,26,27,31, 47]	[11,21,30]	[11,27,47]		[48]	[11,26,27,29,31,47]	[8,12]
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ND: No data

