Improved Diagnosis of Primary *Toxoplasma gondii* Infection in Early Pregnancy by Determination of Antitoxoplasma Immunoglobulin G Avidity

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The ability to discriminate between primary *Toxoplasma gondii* infection acquired in early pregnancy and infection that occurred prior to pregnancy was assessed by an enzyme immunoassay (EIA) to determine the avidity of toxoplasma-specific immunoglobulin G (IgG). The results were compared to those of the Platelia Toxo-IgM EIA and the dye test. The mean IgG avidity of 73 serum samples collected within 20 weeks after the estimated time of infection was 5.9%. Among 26 serum samples showing latent infection (toxoplasma-specific IgG positive and IgM negative) and 56 IgM-positive serum samples with a low dye test titer (<300 IU/ml), the mean avidities were 51.3 and 57.5%, respectively. A total of 72.8% of 92 IgM-positive serum samples with a high dye test titer (>300 IU/ml), suggesting a recent toxoplasma infection, had an IgG avidity of >20%, indicating that the infection started more than 20 weeks earlier. By introducing high IgG avidity as a criterion in the first half of pregnancy to exclude the possibility that toxoplasma infection was acquired during gestation, many women will avoid unnecessary examinations, treatment, and anxiety.

Often, infection by the intracellular parasite *Toxoplasma gondii* either is asymptomatic or results in a clinical disease which is not recognized (8). However, when a pregnant woman undergoes a primary toxoplasma infection, the parasite may be transmitted to the fetus and cause serious damage (19). If the infection is detected, antiparasitic treatment during pregnancy may prevent fetal infection and damage (3, 9, 17, 19).

Toxoplasma infection can be detected by demonstration of specific antibodies (11). If seroconversion is detected in serial serum samples, primary infection is confirmed in the period between the last negative sample and the first positive sample. The presence of toxoplasma-specific immunoglobulin M (IgM) or IgA, or both, in serum are other indicators of recent infection, but these indicators are unreliable, due to differences in the persistence of these antibodies (25). In some patients specific IgA persists for months, while IgM may persist for years following a primary *T. gondii* infection (6, 11). Sabin-Feldman’s dye test has been regarded as the “gold standard” in toxoplasma serology (5, 21). A high dye test value (>300 IU/ml) has been considered to indicate recent infection (2), but the dye test titer may remain high for several months following seroconversion (19). These facts make it difficult to estimate the time of infection when a woman having her first diagnostic test at the beginning of pregnancy has positive toxoplasma-specific IgM or IgA or a dye test value of >300 IU/ml. The specific IgM and IgA and the high dye test titer may have been present before the start of pregnancy, and consequently, the fetus is protected against infection (4).

In 1989 Hedman et al. (7) introduced a new method of detecting recently acquired toxoplasma infection, based on the strength of the binding of specific IgG to multivalent toxoplasma antigen (20). This binding strength, called IgG avidity, was found to be low in the first phase after primary infection but then to increase with time. Later, several investigators (10, 12, 13, 16, 22) have supported the usefulness of IgG avidity determination in the diagnosis of recent *T. gondii* infection. However, in those studies the exact time of acquisition of infection was not given.

We have evaluated a toxoplasma-specific IgG avidity enzyme immunoassay (EIA) on the basis of the results obtained with sequential serum samples from pregnant women in whom the time point of infection could be precisely estimated. The results are compared with those of the dye test and an EIA for the detection of specific toxoplasma IgM for the ability to confirm or exclude *T. gondii* infection in early pregnancy.

(Materials and Methods: Sera. A total of 310 serum samples were included in the study. A total of 114 serum samples were selected among samples from women participating in a national toxoplasma antibody screening project which included 35,940 pregnant women in the period from June 1992 to June 1994 (24). In the same period and until June 1996, 196 additional serum samples were collected from pregnant women not participating in the national project. These sera showed either seroconversion or had detectable toxoplasma-specific IgM, and were thus referred for confirmation to the Toxoplasma Reference Laboratory, National Institute of Public Health, Oslo, Norway.

The sera were allocated to one of five groups according to their toxoplasma immune status.

Serum groups. (i) Sera with rising titers. A total of 107 serum samples were obtained from 23 women (3 to 8 serum samples per woman) with acute *T. gondii* infection on the basis of one of the following criteria. (i) The first serum sample from each woman yielded positive toxoplasma-specific IgM only, while the following samples showed seroconversion for toxoplasma-specific IgG. The estimated time for the acquisition of infection for these women was 1 week prior to collection of the first serum sample (10 women, 56 serum samples). (ii) The first serum sample yielded weakly positive toxoplasma-specific IgG (6 to 20 IU/ml) and positive IgM. The following sera showed fourfold or greater increases in specific IgG and in the dye test titer. For these women acquisition of infection was estimated to be 2 weeks prior to collection of the first sample (13 women, 51 serum samples). Of the 107 serum samples in this group, 80 were collected within 20 weeks after the estimated time of acquisition of infection and 27 serum samples were collected between 20 and 52 weeks after the acquisition of infection.

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As soon as the diagnosis was confirmed, all women were offered antiparasitic treatment according to accepted recommendations (23).

(ii) Seroconversion of indeterminate onset. The first toxoplasma antibody-positive serum sample was obtained from 29 seroconverting women in whom the time of acquisition of the infection could not be exactly estimated. The time that elapsed between the last negative sample and the first positive sample ranged from 13 to 52 weeks, and for 15 of the women 13 to 20 weeks had elapsed. None of the women showed further antibody increases.

(iii) High-titer sera. Ninety-two serum samples were obtained from women with positive toxoplasma-specific IgG and IgM and with high dye test titers (>300 IU/ml). All sera were collected in the first trimester of pregnancy. This antibody pattern indicated a priori a possible acute T. gondii infection (14, 19).

The first toxoplasma antibody-positive serum sample was obtained from 29 seroconverting women in whom the time of acquisition of the infection could not be exactly estimated. The time that elapsed between the last negative sample and the first positive sample ranged from 13 to 52 weeks, and for 15 of the women 13 to 20 weeks had elapsed. None of the women showed further antibody increases.

(iv) Low-titer sera. Fifty-six serum samples were obtained from women with positive toxoplasma-specific IgG and IgM and with low dye test titers (6 to 300 IU/ml). All sera were collected in the first trimester of pregnancy. This antibody pattern indicated a priori a previous T. gondii infection unlikely to have occurred in pregnancy (14, 19). A follow-up sample from each of these women, collected within a month of the time that the first sample was collected, showed stable antibody results (data for the follow-up sera are not included in this report).

(v) Sera from women with latent infections. Twenty-six serum samples were obtained from pregnant women with latent infections with only detectable toxoplasma-specific IgG but no detectable specific IgM.

Serologic tests. All sera were examined for toxoplasma-specific IgG and IgM antibodies by EIA (Platelia Toxo-IgG and Platelia Toxo-IgM; Sanofi Diagnostics Pasteur, Marnes la Coquette, France) (38) at the Toxoplasma Reference Laboratory. Sera with specific IgM antibodies were examined by the dye test (2, 5, 21).

Toxoplasma-specific IgG avidity test. The IgG avidity assay was performed as described by Hedman et al. (7), with minor modifications. The basic test used was the Platelia Toxo-IgG (16). Briefly, each serum sample was analyzed in two fourfold titration rows, with one row (row A) starting at a dilution of 1:50 and the other row (row B) starting at a dilution of 1:200. After 1 h of incubation at 37°C, row A was washed three times with 250 μl of 6 M urea in phosphate-buffered saline containing 0.05% Tween 20 in order to remove low-avidity antibodies from their binding sites. Row B, the control row, was washed three times with the washing solution of the kit, but without urea. During each washing step the microtiter plate was vigorously shaken for 5 min. The following EIA steps, including incubation with conjugate, washing, incubation with substrate, and addition of stop solution, were performed according to the recommendations given by the manufacturer. The optical density (OD) of each well was read in a microtiter plate reader (SLT 340 ATC; SLT-Instruments, Salzburg, Austria) at 492 nm, with 620 nm used as the reference wavelength.

For each serum sample two endpoint titers, one after washing with urea (row A) and one control titer without washing with urea (row B), were calculated as the inverse value of the dilution giving an OD of 0.1 in a semilogarithmic system (OD, arithmetic scale; dilution, logarithmic scale) (7) by the following formula: 

$$titer = -\log_{4} x \times (OD_{1} - 0.1)/(OD_{2} - OD_{1}),$$

where 4 is the dilution factor, OD, is OD at dilution, 0.1 is the cutoff OD, and OD, is the OD at the next higher dilution from dilution. IgG avidity was then calculated as the ratio between the titer in row A and the titer in row B, expressed as a percentage: 

$$avidity = \frac{titer_{row A}}{titer_{row B}} \times 100.$$ 

Figure 1 provides an example illustrating the calculation of the avidities of two serum samples with low and high toxoplasma-specific IgG avidities, respectively. The lower the avidity, the more to the left the titration curve moves after washing with urea. This explains the need to start the titration of row A at a lower dilution than that of row B.

For every 14th serum sample a high-avidity serum sample and a low-avidity serum sample were included as controls. The mean values of 10 repeated tests for these two controls were 60.5% (standard deviation [SD], 7.6%) and 0.3% (SD, 0.2%), respectively.

Statistics. The means, medians, ranges, and SDs of the avidity measurements for the sera from the different groups are given. Probabilities were calculated by assuming a normal distribution (1). Spearman’s rank correlation coefficient (r) was used for comparison (1).

RESULTS

Sera with rising titer. Of the 107 serum samples from the 23 women with rising titers for whom the time of acquisition of infection could be accurately estimated, 22 serum samples yielded a specific IgG EIA titer of <200 without urea treatment. Consequently, for these sera the IgG avidity could not be measured. All these sera were drawn within 5 weeks after the estimated time of acquisition. The results for the remaining 85 serum samples with rising titers are presented in Fig. 2.

The mean avidity for the total group was 7.5%, and the mean avidity was 4.9% for sera collected <20 weeks after the acquisition of infection and 13.1% for sera collected 20 to 52 weeks after the acquisition of infection (Table 1).

Although there was a significant difference between the avidity of sera collected <20 weeks after the acquisition of infection versus that of sera collected 20 to 52 weeks after the acquisition of infection ($P < 0.001$; Mann-Whitney test), indi-
Materials and Methods).

With an acute T. gondii infection, the avidity of the serum sample 3 weeks after acquisition of infection for 85 serum samples from 23 pregnant women was determined. When data concerning sera collected within a year after the last negative serum sample were analyzed, the mean avidity was 10.0% (Table 1). When data concerning sera collected within 20 weeks after acquisition of infection were analyzed, the mean avidity was 11.5%. For the 15 sera collected within 20 weeks after the last negative serum sample, the mean avidity was 10.0% (Table 1).

Sera with seroconversion of indeterminate onset. For the seroconverting women for whom the exact time point of infection was unknown, the mean avidity was 11.5%. For the 15 serum samples taken within 20 weeks after the last negative serum sample, the mean avidity was 10.0% (Table 1).

When data concerning sera collected within a year after the acquisition of infection were lumped together (rising titer and seroconversion sera), only 4 of 114 samples (3.5%) had an avidity of >30%. For the sera taken <20 weeks after the time of acquisition, only 2 of 73 serum samples (2.7%) had an avidity of >20% (Fig. 3). The mean avidity of sera collected <20 weeks after acquisition was 5.9% (median, 3.2%; SD, 6.9%). For one woman whose serum had an IgG avidity of 26.5% 2 weeks after the estimated time of acquisition, the avidity of the next serum sample collected 4 weeks later was 16.1% (Fig. 2).

High-titer sera. For the 92 women with a possible acute infection on the basis of a combination of positive specific IgM and a high dye test titer (>300 IU/ml), the mean avidity was 32.6% (Table 1). These sera covered the whole scale of avidity, from nearly 0 to 100%. Sera from as many as 72.8% (95% CI, 63.7 to 81.9) of them had an IgG avidity of >20%. Twenty-five of the serum samples (27.2%) had an avidity of >20%, and only 19 (20.7%) serum samples had an avidity of <15% (Fig. 3). There was only a weak positive correlation between IgG avidity and IgG values (r = 0.30). Twenty-five of the serum samples (27.2%) had an avidity of >20%, and only 19 (20.7%) serum samples had an avidity of <15% (Fig. 3).

Low-titer sera. For women with positive toxoplasma-specific IgM but a low dye test titer (<300 IU/ml), the mean avidity was 57.7%. One of 56 serum samples (1.7%) from a woman in this group had an avidity of <20% (Fig. 3).

Sera from women with latent infections. The mean avidity for the women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidity of >20% for sera from women with latent infection without detectable toxoplasma-specific IgM was 51.3% (range, 30.6 to 75.4%) (Table 1). According to these results, the probability of having an avidit...
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nearly exclude the possibility that

changed these figures (98.2 and 53.6%, respectively). This collected until 20 weeks after the time of infection only slightly a dye test titer of after infection had a low avidity (1).
The ability of IgG avidity and the dye test to identify precisely when the acute T. gondii infection occurred was further evaluated on the basis of the results obtained with the IgM-positive sera from the group whose sera had rising titers (75 serum samples). Table 2 indicates the proportion of the sera correctly identified as having been collected within 13 or 20 weeks, respectively, and after 13 and 20 weeks, respectively, of the time of infection by using different diagnostic criteria.

Nearly all serum samples (97.8%) collected within 13 weeks after infection had a low avidity (<20%), while only 55.6% had a dye test titer of >300 IU/ml (Table 2). The inclusion of sera collected until 20 weeks after the time of infection only slightly changed these figures (98.2 and 53.6%, respectively). This means that a high-avidity result, but not a low dye test titer, can nearly exclude the possibility that T. gondii infection occurred during pregnancy if the serum sample is collected within the first 20 weeks of gestation.

For sera collected more than 13 weeks after the acquisition of infection, 53.3% had an avidity of >10% and only 23.3% an avidity of >20%. The dye test titer was <300 IU/ml for 60% of these sera. This means that neither a low-avidity result nor a high dye test titer could confirm that the infection had occurred within the previous 13 weeks (or 20 weeks). However, for 94.7% of the sera collected more than 20 weeks after infection, either the avidity was >10% or the dye test titer was <300 IU/ml. This means that if both the avidity is <10% and the dye titer is >300 IU/ml, the infection most likely occurred within the 20 weeks prior to the collection of the serum sample (Table 2).

**Effect of antiparasitic treatment.** Of the 85 serum samples examined in the rising titer group, 46 (54%) were collected before and 39 (46%) were collected after the start of antiparasitic treatment of the women. No significant difference in mean avidity was found between sera from treated and untreated patients.

**DISCUSSION**

The ideal situation for the diagnosis of T. gondii infection in pregnancy would be to have an antibody-negative serum sample collected at the very beginning of pregnancy or preferably before conception. Usually, this is not possible. When routine testing for toxoplasma antibodies in pregnancy is performed, the first serum sample is usually taken at the first antenatal health care visit confirming pregnancy. In Norway this usually occurs between the 8th and 13th weeks of gestation. If seroconversion is the only accepted criterion for the diagnosis of T. gondii infection, these first gestational weeks (25 to 30% of the total duration of the pregnancy) will not be covered by a serological screening program aimed at the detection of congenital toxoplasmosis (15). The test or the combination of tests to be used in such an antenatal screening program should ideally be able to determine if the infection occurred after conception, i.e., within 8 to 13 weeks before the day of sampling.

Our study of serial blood samples from acutely infected pregnant women indicates that in the presence of toxoplasma-specific IgM-positive serum samples, the additional presence of a high dye test titer (>300 IU/ml) is insufficient to identify an acute infection within the previous 13 weeks, and a value of <300 IU/ml is correspondingly insufficient to exclude such an infection. Therefore, when using the presence of specific IgM and a high dye test titer as criteria for identifying T. gondii infection in early pregnancy, some acute infections will not be detected. On the other hand, many women will be falsely identified as possibly infected in pregnancy and unnecessarily undergo diagnostic amniocentesis and antiparasitic treatment.

Earlier studies have indicated that low IgG avidity is a marker of recent infection (7, 10, 12, 13, 16, 22). However, those studies either included a limited number of serum samples (7, 10, 12) or correlated the results to the time that had elapsed, not from the acquisition of infection until the collection of the serum sample but from the time when the first sample showing toxoplasma antibody was collected, which may be several weeks after the time of infection (13, 16). The first symptoms of lymphadenopathy, used as a point of reference, may also be detected some time after the acquisition of infection (10, 22). Our study showed that a low IgG avidity in some cases persisted for more than 20 weeks after the time of acquisition. Thus, a low IgG avidity could not accurately identify an acute infection within the previous 13 to 20 weeks of pregnancy.

On the other hand, only 2 of 73 serum samples (2.7%) sampled within 20 weeks after infection had an IgG avidity of >20%. This means that an IgG avidity of >20% can be used to exclude an acute infection. Lappalainen et al. (13) found that

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**TABLE 2.** Proportion of sera correctly identified as having been collected within or after 13 and 20 weeks of the time of infection, using different diagnostic criteria

<table>
<thead>
<tr>
<th>Diagnostic criterion</th>
<th>Sera collected within time after infection</th>
<th>Sera collected after time after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 wk (n = 45)</td>
<td>20 wk (n = 56)</td>
</tr>
<tr>
<td>Dye test titer, &gt;300 IU/ml</td>
<td>55.6</td>
<td>53.6</td>
</tr>
<tr>
<td>IgG avidity, &lt;10%</td>
<td>86.7</td>
<td>82.1</td>
</tr>
<tr>
<td>IgG avidity, &lt;15%</td>
<td>93.3</td>
<td>91.1</td>
</tr>
<tr>
<td>IgG avidity, &lt;20%</td>
<td>97.8</td>
<td>98.2</td>
</tr>
<tr>
<td>&lt;10% and &gt;300 IU/ml</td>
<td>51.1</td>
<td>46.4</td>
</tr>
<tr>
<td>&lt;15% and &gt;300 IU/ml</td>
<td>53.3</td>
<td>51.8</td>
</tr>
<tr>
<td>&lt;20% and &gt;300 IU/ml</td>
<td>55.6</td>
<td>53.6</td>
</tr>
</tbody>
</table>

a The results are based on 75 toxoplasma-specific IgM-positive serum samples from 23 women for whom the time of infection could be estimated (rising titer group; see text).
b Values in the columns indicate the percentage of serum samples in each week group that fulfilled the indicated diagnostic criteria.

Combination of avidity and dye test titer.

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This page contains a detailed analysis of the diagnosis of T. gondii infection in early pregnancy, focusing on the use of IgG avidity and dye test titers to determine the timing of infection. The table provides data on the proportion of sera that were correctly identified as having been collected within or after 13 and 20 weeks of the time of infection, using different diagnostic criteria. The study highlights the importance of considering both IgG avidity and dye test titers in the diagnosis of toxoplasmosis during pregnancy.
the predictive value of a high-avidity test result for *T. gondii* infection within the previous 5 months among 75 seropositive pregnant women was 100%.

Among 92 routinely collected serum samples from pregnant women with toxoplasma-specific IgM and a high dye test titer (>300 IU/ml), 72.8% showed an IgG avidity of >20%. This means that for nearly three-quarters of the women who by previous criteria were suspected of having an acute infection, the acquisition of toxoplasma in early pregnancy could be excluded on the basis of the results for a single sample collected in the first trimester, without the need for further examinations and anxiety.

In the low-titer group, 1 of 56 serum samples (1.8%) had an IgG avidity of below 20%; specifically, the result was 17.7%. For sera from the latent infection group, the probability of having an avidity of <20% was as low as 0.4%. Thus, the avidity assay of sera supported the conclusion of a latent infection for the women in either of these two groups.

Our results strongly support those of Lecolier and Pucheu (16), who conclude that the toxoplasma-specific IgG avidity test should be performed with the first blood sample collected in early pregnancy to exclude acute infection during gestation (16). By confirming latent infection on the basis of a high IgG avidity, the need to collect a second serum sample for examination for a possible antibody increase seems to be eliminated.

The poor ability of the dye test result for a single serum sample to confirm a primary infection (Table 2) is explained by the fact that sera from many women with acute *T. gondii* infection never reach a peak value of 300 IU/ml or more by this test. Acutely infected women whose sera have a low dye test titer may therefore erroneously be diagnosed as having a latent infection instead of an acute infection occurring in pregnancy.

A lower avidity indicated a higher probability of an acute infection. In addition, if the dye test titer was high, the results indicated that a primary toxoplasma infection had been acquired within the previous 20 weeks from the time that the serum sample was collected (Table 2).

Sensini et al. (22) concluded that antiparasitic treatment may postpone the maturation of toxoplasma-specific IgG avidity. In our study nearly all pregnant women with primary toxoplasma infection were treated with antibiotics. Therefore, apart from the first samples from each woman, which were collected before treatment, the avidity of the follow-up samples might have been higher without treatment. We did not find any significant difference in avidity between sera collected after treatment and sera collected before treatment, but the number of serum samples in each group to be compared was low. However, if treatment delays maturation of toxoplasma IgG antibodies and therefore postpones the development of high-avidity IgG, the diagnostic specificity of the avidity test for nontreated women should be higher than that calculated from our results. Whether the ability to exclude acute infection on the basis of high IgG avidity is simultaneously significantly reduced needs further studies.

The IgG avidity assay. In the previous published works describing the detection of toxoplasma-specific IgG avidity, different antigen preparations have been used. The quality of these antigens may vary, and a comparison of the different avidity tests that have been performed has not been done. Like Lecolier and Pucheu (16), we used a commercially available kit in which the membrane protein p30 is the predominant antigen.

The method of calculating the toxoplasma-specific IgG avidity also differs among the previous studies. We used the method of Hedman et al. (7), including titration of each sample and calculation of the titers, with and without urea washing, in relation to a defined cutoff value. An easier method is to test only one serum dilution and compare the OD of the urea-washed well with that of the control well directly, an approach used by Holliman et al. (10). In our preliminary experiments we encountered difficulties with this method, mainly because the avidity seemed to vary depending on the total amount of toxoplasma-specific IgG in the test well. Lecolier and Pucheu (16) encountered the same problem and tried to solve this by testing each serum sample at an individual dilution determined beforehand by a standard Platelia Toxo-IgG analysis. In this manner they tried to get an OD result for the control well for all sera close to but above the cutoff value. In fact, this is a kind of titration which was used in a direct comparison of the OD of the urea-treated well to that of the control well. Joynson et al. (12) chose titration with comparison of titers, but selected the cutoff not as a fixed OD but as 50% of the maximum absorbance for the control titration row.

It is clear that these different ways of calculating the results are not directly comparable even if all the methods finally express the results as percent IgG avidity. To be able to compare results between laboratories in the future, there is a need for an agreement on how to perform the test and calculate the avidity.

Conclusions. By introducing the toxoplasma-specific IgG avidity assay with specific IgM-positive samples collected in the first half of pregnancy, the diagnostic routine for the detection of *T. gondii* infection in early pregnancy is improved. The main achievement is the ability to exclude infection in pregnancy for many women who otherwise, on the basis of a positive specific IgM result and/or a high dye test titer, would have been identified as having a recent infection. These women will be saved from unnecessary anxiety and additional examinations and treatment. The test is easy to perform at any medical microbiological laboratory, but there is a need for standardization of the method.

REFERENCES


