CVI Journals.ASM.org

Toxoplasma gondii Recombinant Antigens as Tools for Serodiagnosis of Human Toxoplasmosis: Current Status of Studies

Lucyna Holec-Gąsior

Gdańsk University of Technology, Chemical Faculty, Department of Microbiology, Gdańsk, Poland

***Toxoplasma gondii* is a parasitic protozoan which is the cause of toxoplasmosis. Although human toxoplasmosis in healthy adults is usually asymptomatic, serious disease can occur in the case of congenital infections and immunocompromised individuals. Furthermore, despite the exact recognition of its etiology, it still presents a diagnostic problem. Diagnosis of toxoplasmosis is mainly based on the results of serological tests detecting anti-*T. gondii*-specific antibodies in the patient's serum sample. The specificities and sensitivities of serology tests depend mostly on the diagnostic antigen(s) used. Most of the commercial serological kits currently available are based on *Toxoplasma* lysate antigens (TLAs). In recent years, many studies showed that recombinant antigenic proteins of *T. gondii* may be an alternative source of antigens which are very useful for the serodiagnosis of toxoplasmosis. This article presents a review of current studies on the application and usefulness of different *T. gondii* recombinant antigens in serological tests for the diagnosis of human toxoplasmosis.**

Toxoplasmosis is a parasitic disease caused by the intercellular protozoan parasite *Toxoplasma gondii*, which can infect humans and warm-blooded animals (1). In healthy individuals, a primary infection with *T. gondii* usually causes relatively mild flu-like symptoms, whereas in immunocompromised patients, it can cause opportunistic life-threatening infections. Moreover, in pregnant women, toxoplasmosis may cause serious problems because transplacental transmission can occur and lead to abortion, stillbirth, or neonatal malformations (2). Overall, about a third of mothers with primary infection give birth to an infant with toxoplasmosis (2). These facts emphasize the importance of being able to make an accurate distinction between primary and chronic infection or reactivation, especially during pregnancy.

A diagnosis of toxoplasmosis can be established by the isolation of *T. gondii* from blood or body fluids, demonstration of the parasite in tissues, detection of specific nucleic acids with DNA probes, or by carrying out serologic tests in order to detect *T. gondii*-specific immunoglobulins synthesized by the host in response to infection. Currently, routine diagnosis of toxoplasmosis relies mainly on the use of various serological tests to detect specific antibodies in the serum samples of infected patients. The presence of a recent infection can be determined by detecting seroconversion of immunoglobulin M (IgM) or IgG antibodies, a substantial increase in IgG antibody titer, or a *Toxoplasma* serologic profile compatible with acute infection (using *Toxoplasma* serodiagnostic tests, including an IgG avidity test) in sequential serum samples of infected individuals (3, 4). However, this procedure bears limitations in estimating the time of *T. gondii* infection due to the fact that, in most cases, low IgM titers persist long after the acute phase of disease (5). Most commercial serological kits use native antigens prepared from tachyzoites grown in mice and/or tissue culture. The methods of producing these antigens may also vary significantly between laboratories. It is important to know that the antigen(s) obtained from tachyzoites may contain various nonparasitic materials from culture media and eukaryotic host cells. Serological tests based on tachyzoite antigenic extracts are thus difficult to standardize and frequently provide insufficient specificity and results which are too inconsistent for accurate differentiation between newly acquired infections and those acquired long before conception (6–8). One approach to improving these tests is to replace the native antigens with recombinant proteins. The major advantages of recombinant antigens for the diagnosis of *T. gondii* infections are as follows: (i) the precise antigen composition of the test is known, (ii) more than one defined antigen can be used, and (iii) the method can easily be standardized. In addition, selected antigens that are characteristic for the acute or chronic stages of the infection could serve as a tool to discriminate between the two stages. Moreover, obtaining homogenic recombinant specimens of antigenic proteins by molecular biology methods makes it possible to solve not only the biohazard problem but also the issue of time and labor consumption, all of which accompany the production of native antigen.

This article presents the current status of the application of *T. gondii* recombinant antigens in the diagnosis of human toxoplasmosis, focusing on the use of specific molecular markers, mixtures of proteins, and the new generation of diagnostic tools, which is to say, chimeric antigens, in serological tests.

T. GONDII RECOMBINANT ANTIGENS

In the past 35 years, several dozen genes encoding *T. gondii* proteins have been cloned into bacterial and eukaryotic expression systems, as follows: the surface antigens SAG1 (P30) (9–12), SAG2 (P22) (12–15), SAG3 (P43) (16), and P35 (15); the dense granule antigens GRA1 (P24) (11, 17), GRA2 (P28) (18–22), GRA4 (23, 24), GRA5 (25), GRA6 (P32) (15, 26, 27), and GRA7 (P29) (11, 28–31); the rhoptry antigens ROP1 (P66) (20) and ROP2 (P54) (32–34); and B10 (P41) (35), MAG1 (36), and MIC1 (37). Many of these recombinant antigens have been used for the detection of *T. gondii*-specific antibodies in human serum samples (38). Furthermore, several previous studies have found that recombinant antigens improve the serological diagnosis of toxoplasmosis (15,

Published ahead of print 19 June 2013

Address correspondence to Lucyna Holec-Gąsior, holec@pg.gda.pl.

doi:10.1128/CVI.00117-13

TABLE 1 Recombinant antigens of *T. gondii* as molecular markers of the toxoplasmosis phase

Antigen	Expression system/protein[a]	Diagnostic test	No., source, and toxoplasmosis phase[b] of seropositive sera tested	Results	Reference
GRA6	Recombinant protein with GST domain	IgG ELISA	193 total, 61 acute, 132 chronic	Sensitivity, 86% for acute-phase sera; specificity between presence and absence of acute infection, 99.6%	27
P35	Recombinant protein with GST domain	IgM ELISA	53 total, 20 acute, 33 chronic	Sensitivity, 90% for acute-phase sera; no chronic sera were positive	49
P35	Recombinant protein with CKS[c]	IgG ELISA	91 total from 80 pregnant women, 41 acute, 50 chronic	Sensitivity, 85.3% for acute-phase sera, 8% for chronic sera	50
GRA4	Recombinant protein with His tag domain	IgG ELISA	36 total, 12 acute, 22 chronic	Sensitivity, 58.3% for acute-phase sera, 18.2% for chronic sera	51
GRA7				Sensitivity, 75% for acute-phase sera, 36.3% for chronic sera	
MIC3	Recombinant protein with GST domain	IgG avidity	121 total from 80 pregnant women with seroconversion	Low avidity of IgG antibodies in sera collected within 2 mo after infection	52
GRA7	Recombinant protein with His tag domain	IgG ELISA	117 total, 45 acute, 72 chronic	Sensitivity, 95.9% for acute-phase sera, 68.9% for chronic sera	43
GRA6	Recombinant protein with His tag domain	IgG ELISA	90 total, 33 acute, 57 chronic	Sensitivity, 93.9% for acute-phase sera, 63.1% for chronic sera	15
P35				Sensitivity, 86.7% for acute-phase sera, 54.5% for chronic sera	
MAG1	Recombinant protein with His tag domain	IgG ELISA	117 total, 37 acute, 80 chronic	Sensitivity, 97.3% for acute-phase sera, 7.5% for chronic sera	36
GRA2	Recombinant protein with TRX and His tag domains	IgG ELISA	59 total from pregnant women in France, 24 acute, 35 chronic	Sensitivity, 95.8% for acute-phase sera, 67.7 % for chronic sera	53
			46 total from pregnant women in Iran, 18 acute, 28 chronic	Sensitivity, 100% for acute-phase sera, 71.4% for chronic sera	
SAG2A	Recombinant protein with His tag domain	IgG ELISA	60 total, 30 acute, 30 chronic	Sensitivity, 90% for acute-phase sera, 67% for chronic sera	54
GRA6	Recombinant protein with His tag domain	IgG ELISA	58 total, 24 acute (seroconversion during 4 mo prior to sampling), 34 chronic	For cutoff value[d] of mean + 2 SD, sensitivity was 95.8% for acute-phase sera, 44.1% for chronic sera; for cutoff value[d] of mean + 3 SD, sensitivity was 87.5% for acute-phase sera, 5.9% for chronic sera	55
ROP1	Recombinant protein with His tag domain	IgG ELISA	127 total, 37 acute, 90 chronic	Sensitivity, 94.6% for acute-phase sera, 15.5% for chronic sera	20
GRA2				Sensitivity, 100% for acute-phase sera, 22.5% for chronic sera	
GRA7	Recombinant protein with His tag domain	IgG immunoblot	40 total, 20 acute, 20 chronic	Sensitivity, 100% for acute-phase sera, 40% for chronic sera	12

[a] All recombinant proteins were produced in *E. coli*.
[b] Sera were grouped according to toxoplasmosis phase (acute or chronic).
[c] CKS, CTP:CMP-3-deoxy-D-manno-octulosonate cytidylyl transferase.
[d] The cutoff values were set on the mean absorbency readings of sera from seronegative individuals.

25, 34, 39–48). However, it is not only the improvement of *T. gondii* diagnosis with the use of recombinant antigens that is very important but, also, improvement in differentiating between the acute and chronic phase of toxoplasmosis. The determination of the stage of *T. gondii* infection is important for pregnant women because primary infection during pregnancy can put the child at risk of serious medical problems. The accurate diagnosis of acute infection in pregnant women is thus critical for the clinical management of both the mother and her fetus. For these reasons, it is vitally important to investigate better diagnostic methods for distinguishing between recently acquired infections and those that occurred prior to conception.

A new possibility in *T. gondii* serodiagnosis is the selection and use of molecular markers, which is to say, proteins characteristic of the parasite's tachyzoite or bradyzoite stage that are able to recognize the specific antibodies from acute or chronic human sera (Table 1). In recent years, many papers have reported very promising results, showing that, in many cases, the use of a specific recombinant protein identifies the phase of the disease during the testing of a single sample of serum. Table 1 presents different *T. gondii* recombinant proteins that have shown stronger reactions with sera from patients with acute toxoplasmosis. Thus, these results indicate that properly selected recombinant antigens are suitable for the investigation of the acute phase of disease. Moreover, in the diagnosis of acute infection versus chronic infection, determining the avidity index of IgG antibodies is very important (56–59). The application of *T. gondii* recombinant antigens in an IgG avidity assay presents completely new possibilities. Against se-

lected recombinant proteins, IgG avidity matures at a different rate than the *Toxoplasma* native antigens. As a result, it is possible to improve IgG avidity assays by better discrimination of the sera on the basis of the length of time that the *T. gondii* infection has been present. In 2000, Marcolino et al. (60) selected proteins P16, P32, P38, P40, P43, P54, P60, P66, and P97 as potentially useful in an avidity assay to discriminate between phases of toxoplasmosis, while in 2003, Beghetto et al. (52) showed that MIC3 may be used as a molecular marker that discriminates on the basis of avidity results between sera from patients infected with *T. gondii* more and less than 2 months previously (Table 1). Moreover, in 2005, Pfrepper et al. (45) constructed an avidity test in which GRA7, MAG1, and SAG1 antigens were applied. In this study, the recom-Line *Toxoplasma* IgG strip test (Mikrogen GmbH, Germany) showed low avidity for recombinant GRA7 within the first month of infection, while low avidity for MAG1 and SAG1 was found within a postinfection period of 3 months. In 2008, Sickinger et al. (61) developed the ARCHITECT toxo IgG and IgG avidity assays as a fully automated panel for immune status determination and acute infection exclusion. In these tests, the tachyzoite-specific surface antigen P30 (SAG1) and P35 (GRA8) proteins were used. The IgG avidity assay detected 100% (124/124) of acute-phase specimens (<4 months after infection) as low avidity, whereas the commercially available Vidas toxo IgG avidity assay (bioMérieux, France) detected 98.9% (89/90) as low avidity. ROP1 recombinant antigen was also applied in an IgG avidity test (62), where specific low-avidity antibodies were detected in most of the sera from individuals with acute toxoplasmosis, while the absence of specific antibodies or high-avidity antibodies was detected in sera from patients with chronic infection. Furthermore, in 2010, Elyasi et al. (63) showed better clinical usefulness for an IgG avidity assay based on recombinant GRA6 than for the Euroimmun avidity test (Euroimmun, Lübeck, Germany) for the exclusion of recent infection, occurring less 4 months previously, in pregnant women. Results for a selected mixture of recombinant proteins, namely, GRA7, SAG1, and GRA1, were also published (64), showing that IgG avidity maturation against this mixture is different from that received against *Toxoplasma* lysate antigen (TLA). Therefore, the use of recombinant proteins in IgG avidity assays has great potential and offers new opportunities for the diagnosis of toxoplasmosis. Avidity assays based on whole-cell *T. gondii* antigen detect low- or borderline-avidity antibodies in many patients with the chronic phase of disease (59, 65, 66), whereas affinity maturation of antibodies against single epitopes represented by properly selected recombinant antigens might follow a different pattern and, thus, distinguish better between acute and chronic toxoplasmosis. Recombinant antigens could therefore enhance the clinical usefulness of avidity assays in order to determine more accurately when *T. gondii* infection occurred. In addition, the standardization of an avidity test based on recombinant antigens, which have a more-constant quality than TLAs prepared from parasites grown either in a cell culture or in the peritoneal cavities of mice, should be easier.

In summarizing the information presented in this section, it must be concluded that recombinant immunoassays using specific molecular markers appear to be a useful addition to the current methods used for the serodiagnosis of acute toxoplasmosis. Moreover, Redlich and Müller (27) reported that an assay using a recombinant product in the form of the glutathione *S*-transferase (GST)-GRA6 antigen can provide important additional information about the stage of *T. gondii* infection in patients with IgM persistence.

THE MIXTURE OF RECOMBINANT PROTEINS

In most studies, recombinant antigens were coated on enzyme-linked immunosorbent assay (ELISA) plates alone. However, in some cases, they were coated in various combinations consisting of two or three proteins. The diagnostic utility of two recombinant *T. gondii* proteins, H4/GST and H11/GST, mixed together as an antigen preparation in an ELISA for the detection of *T. gondii*-specific IgM was shown for the first time by Johnson et al. (67) in 1992. Their results revealed higher sensitivity (81.3%) of the IgM ELISA with a combination of the two proteins than was previously obtained with H4/GST and H11/GST separately (54% and 61%, respectively) (68). A mixture of GRA7, GRA8, and ROP1 recombinant proteins was subsequently reported as an antigen preparation for the detection of IgM antibodies in human sera (Table 2) (41). Another interesting combination for the detection of IgG-specific antibodies, based on recombinant GRA7 together with Tg34AR (ROP2 C-terminal fragment), was reported by Jacobs et al. (40). In this particular case, while each separate ELISA had a sensitivity of 81% and 88%, respectively, the combination of the two proteins increased the sensitivity to 96%. Taken together, these data demonstrated the potential of using two or three complementary recombinant antigens in order to reach a sensitivity comparable to that obtained with a crude antigen preparation. In 2000, Lecordier et al. (42) showed that the sensitivity of IgG ELISAs with the single recombinant antigens GRA1 and GRA6-Nt was low (68% and 96%, respectively). They also found that comparison of the optical density values for each serum sample revealed that GRA1 may complement GRA6-Nt to reach an overall sensitivity of 98%. Hence, these proteins could be used together as a mixture of antigens for the serodiagnosis of toxoplasmosis. Furthermore, several mixtures of recombinant proteins were proposed for the detection of IgG antibodies against *T. gondii*. They were GRA7, GRA8, and SAG1 (41); GRA7, GRA8, SAG2, and H4 (69); SAG1, GRA1, and GRA7 (43); P35 (GRA8), SAG2, and GRA6 and MIC1ex2, MAG1, and MIC3 (37); and SAG1 and GRA5 mixed with MAG1 or GRA2 or ROP1 (25) (Table 2). The results presented in the studies cited above are consistent in that all the researchers found that combining complementary recombinant *T. gondii* antigens in the same immunoassay improves the relative sensitivity of the test. Moreover, the above-mentioned combinations of recombinant antigens include one consisting of GRA7, GRA8, SAG2, and H4 proteins that was suggested as being useful for differentiating between a recently acquired infection and one acquired in the distant past (69). Most of the cocktails under discussion were found to be antigenic preparations which can detect specific IgG in the sera of infected individuals with a sensitivity that is the same or similar to the native antigen of *T. gondii*. These combinations of recombinant proteins can therefore replace the tachyzoite soluble extract in serologic tests. However, the detection of IgG antibodies from different stages of the disease requires an optimum combination of well-proportioned antigens that are highly reactive, such as SAG1, and which are specific molecular markers for different phases of toxoplasmosis, for example, MAG1, GRA2, or ROP1 for the acute stage or GRA5 for the chronic (25). Thus, a well-advised and precise selection of mixture components is crucial to obtaining a preparation useful for diagnostic application.

TABLE 2 Combinations of *T. gondii* recombinant antigens for the diagnosis of toxoplasmosis

Antigen mixture	Expression system/protein[a]	Diagnostic test	No., source, and toxoplasmosis phase[b] of seropositive sera tested	Results	Reference
P29 (GRA7), P30 (SAG1), P35 (GRA8)	Recombinant proteins with CKS[c] domain	IgG ELISA	247 total, 92 chronic, 88 acute, 53 seroconverted	Sensitivity, 98.4%; specificity, 95.7%	41
P29 (GRA7), P35 (GRA8), P66 (ROP1)		IgM ELISA	142 total, 88 acute, 53 seroconverted	Sensitivity, 93.1%; specificity, 95.0%	
P22 (SAG2), P25, P29 (GRA7), P35 (GRA8)	Recombinant proteins with MBP domain (P22, P25, P29) and GST domain (P35)	IgG ELISA	90 total from pregnant women, 20 acute, 70 chronic	Sensitivity, 90% for acute-phase sera, 1.4% for chronic sera; specificity, 97% for acute-phase sera; useful for differentiation of acute- from chronic-phase toxoplasmosis	68
SAG1, GRA1, GRA7	Recombinant proteins with His tag domain	IgG ELISA	241 total, 117 acute, 124 chronic	Sensitivity, 100% for acute-phase sera, 91.1% for chronic sera	43
P35 (GRA8), SAG2, GRA6	Recombinant proteins with His tag domain	IgG ELISA	72 chronic sera	Sensitivity, 88.9%; specificity, 100%	37
MIC1ex2, MAG1, MIC3				Sensitivity, 94.4%; specificity, 100%	
MAG1, SAG1, GRA5	Recombinant proteins with His tag domain	IgG ELISA	189 total, 27 acute, 18 postacute, 144 chronic	Sensitivity, 92.6%; specificity, 100%	25
GRA2, SAG1, GRA5				Sensitivity, 93.1%; specificity, 100%	
ROP1, SAG1, GRA5				Sensitivity, 94.2%; specificity, 100%	

[a] All recombinant proteins were produced in *E. coli*.
[b] Sera were grouped according to toxoplasmosis phase (acute, postacute, or chronic).
[c] CKS, CTP:CMP-3-deoxy-D-manno-octulosonate cytidylyl transferase.

In view of the information set out in this section, it seems that the development of appropriate mixtures of recombinant proteins is a good approach in the diagnosis of toxoplasmosis. In some cases, single antigens may have low sensitivity in the detection of specific antibodies in serum samples from infected individuals. This may be due to the lack of specific antibodies against a given protein in the patient's serum. Therefore, the use of a mixture of recombinant antigens is better for identification purposes than a single protein because the application of two or several recombinant antigens could improve the sensitivity of ELISAs.

CHIMERIC ANTIGENS AND MULTIEPITOPE PEPTIDES

Chimeric products are a new kind of diagnostic tool for the detection of anti-*T. gondii*-specific antibodies in human serum samples. A single recombinant chimera contains different immunoreactive epitopes from various *T. gondii* antigens which have been properly selected. Generally, these immunoreactive epitopes are hydrophobic segments of antigen, and very often, they are fragments that are well exposed on the protein surface. In the past, a variety of computing methods for the prediction of epitopes have been developed (70). Most of them utilize the physical and chemical properties of individual amino acids to predict structural and functional properties of peptide chains suggesting the localization of epitopes. Moreover, in recent years, new tools have been developed to predict immunoreactive protein fragments. In 2012, Maksimov et al. (71) showed that the use of a bioinformatic prediction method in combination with a peptide microarray is a powerful tool for the selection and analysis of *T. gondii* epitopes as candidate antigens for serological diagnosis. Furthermore, several experimental approaches may be used to identify epitopes, including phage display of cDNA libraries, epitope mapping, and reactivity with monoclonal antibodies (72–74). The combination of carefully selected epitopes from proteins of different stages of the *T. gondii* life cycle is an optimal strategy for overcoming the antigen complexity of the parasite. Thus, the chimeric protein may be a more immunodominant antigen than the original antigens. Moreover, a major advantage of using a chimeric antigen for antibody detection rather than the existing commercial assays and tests based on a combination of recombinant products would be a more standardized antigen. Chimeric antigens are a new generation of recombinant products which have the potential to replace the native antigen(s) received from lysed whole parasites. To date, only a few studies have shown the usefulness of this kind of proteins for the serological diagnosis of *T. gondii* infection (Table 3). In 2006, Beghetto et al. (46) found that two chimeric antigens, GST-EC2 and GST-EC3, improve the serological diagnosis of toxoplasmosis both in adults with an acquired infection and in infants born to mothers with a primary *T. gondii* infection (Table 3). In addition, those authors showed that, compared with the ability to diagnose congenital infection by using standard assays, IgM ELISAs with recombinant antigens (Rec-ELISAs) based on GST-EC2 and GST-EC3 proteins improve the ability to diagnose congenital toxoplasmosis postnatally. Their research demonstrated that the IgM Rec-ELISA found 70% of serum samples from infants with congenital toxoplasmosis to be positive, whereas the commercially available assays used (ELFA-IgM [bioMérieux, France] or ETI-TOXOK-M [DiaSorin, Italy]) found positive results for only 35% of the infected infants (46). Furthermore, different chimeric proteins have also been shown to be very effective in detecting specific anti-*T. gondii* antibodies in human serum samples (Table 3) (75–79). In 2012, Holec-Gąsior et al. (76) showed that the sensitivity of the IgG ELISA for the MIC1-MAG1 chimeric protein was almost as high as that for the *Toxoplasma* lysate antigen (TLA), 90.9% and 91.8%, respectively, whereas the sensitivities of IgG ELISAs for the mixture of two recombinant proteins (rMAG1 and rMIC1ex2) or for single recombinant antigens were definitely lower, 69.1% for the mixture, 75.5% for rMIC1ex2, and

TABLE 3 *T. gondii* recombinant chimeric antigens and multiepitope peptides for the serodiagnosis of toxoplasmosis

Chimeric antigen(s)/ multiepitope peptide(s)	Antigenic regions (aa)	Expression system and recombinant protein	Diagnostic test	No., source, and toxoplasmosis phase[a] of seropositive sera tested	Results	Reference
GST-EC2 and GST-EC3	157–235 of MIC2, 234–307 of MIC3, 182–312 of SAG1 and 36–134 of GRA3, 24–102 of GRA7, 37–263 of M2AP	Recombinant proteins with GST domain produced in *E. coli*	IgM ELISA or IgG ELISA	50 from adults with acquired *T. gondii* infection	IgM ELISA, sensitivity, 98% (49/50) for GST-EC2 antigen, 84% (42/50) for GST-EC3 antigen	46
				20 from infants born to mothers with primary toxoplasmosis	IgM ELISA, sensitivity, 70% (14/20) for GST-EC2 antigen, 50% (10/20) for GST-EC3 antigen	
				100 from adults with acquired *T. gondii* infection	IgG ELISA for GST-EC2 and GST-EC3 antigens, sensitivity and specificity, 100%	
SAG1/2	1–336 of SAG1, 1–186 of SAG2 (whole proteins)	Recombinant protein with His tag domain produced in *P. pastoris*	IgG/IgM Western blotting	110 total, 20 early acute, 20 acute, 20 chronic	Sensitivity and specificity, 100%	75
MIC1-MAG1	25–182 of MIC1, 30–222 of MAG1	Recombinant protein with His tag domain produced in *E. coli*	IgG ELISA	110 total, 26 acute, 17 postacute, 67 chronic	Sensitivity, 100% for acute-phase and postacute-phase sera, 85.1% for chronic sera, 90.9% overall; specificity, 100%	76
MIC1-MAG1-SAG1	25–182 of MIC1, 30–222 of MAG1, 49–198 of SAG1	Recombinant protein with His tag domain produced in *E. coli*	IgG ELISA	162 total, 47 acute, 19 postacute, 96 chronic	Sensitivity, 100% for acute-phase and postacute-phase sera, 96.9% for chronic sera, 98.1% overall; 100% specificity	77
MEP	239–318 of SAG1, 109–118 of SAG2, 347–356 of SAG3	Recombinant protein with His tag and Trx tag domains produced in *E. coli*	IgG ELISA	108 total, 32 acute, 76 chronic	Sensitivity, 87.5% for acute-phase sera, 97.4% for chronic sera, 94.4% overall; 100% specificity	78
			IgM ELISA	32	Sensitivity, 96.9%	
			IgG ELISA	126 total from pregnant women, 58 acute, 68 chronic	Sensitivity, 25.9% for acute-phase sera, 97.1% for chronic sera, 96.6% overall	79
			IgM ELISA	58 from pregnant women	Sensitivity, 96.6%; specificity, 100%	

[a] Sera were grouped according to toxoplasmosis phase (acute, postacute, or chronic).

60% for rMAG1. Thus, the MIC1-MAG1 recombinant chimeric antigen has the potential to replace the TLA in the IgG ELISA. Furthermore, the same research team also developed the MIC1-MAG1-SAG1 chimeric protein containing immunodominant regions from three *T. gondii* antigens (77), which yielded better results than the chimeric antigen containing only two fragments from the MIC1 and MAG1 proteins (76) (Table 3). The results of this study showed that, in the case of antigen construction for diagnostic utility, the rational selection of protein fragments is of great importance. The addition of the fragment of surface antigen (SAG1), one of the parasite's most-immunogenic proteins, to the chimeric antigen resulted in an increase in the reactivity with specific IgG antibodies from the sera of patients with chronic toxoplasmosis. Another diagnostic approach, applied by Dai et al. (78), was related to the identification of immunodominant B-cell epitopes within the six *T. gondii* proteins SAG1, SAG2, SAG3, GRA5, GRA6, and P35. Three recombinant epitopes, which were cloned from SAG1, SAG2, and SAG3, were strongly recognized by *T. gondii*-positive human sera. For this reason, the authors developed a recombinant multiepitope fusion peptide (rMEP) composed of these three antigenic determinants, which was tested with diverse groups of human sera in IgG and IgM ELISAs (78, 79). The results showed that the rMEP was effective in discriminating between recent and past infection (Table 3). The authors also showed that the rMEP-based assay, when compared with two commercially available ELISA kits (ELISA classic *T. gondii* IgG and IgM tests; Serion, Würzburg, Germany), has similar detection efficiencies (93.2% and 95.7% for the detection of IgG and IgM antibodies, respectively). These results suggest that rMEP recombinant multiepitope peptide antigens may be useful for the serologic diagnosis of toxoplasmosis in pregnant women.

To summarize, the use of chimeric antigens and multiepitope peptides in the serodiagnosis of *T. gondii* infection reveals new diagnostic possibilities. These recombinant products may not only facilitate the development of more-reliable and more-consistent test systems but may also allow the development of new tests capable of discriminating recently acquired infections from those acquired in the more-distant past. However, an antigenic preparation useful for differentiation of the acute and chronic phases of disease should contain various immunodominant regions from specific molecular markers recognizing early or late anti-*T. gondii* antibodies in human sera.

CONCLUSION

In the quest for suitable diagnostic tools for the detection of *T. gondii* infection and differentiation between the phases of toxoplasmosis, numerous recombinant antigens of the parasite have been reported and a number of them have already been evaluated in ELISA and Western blot tests for the recognition of anti-*T. gondii* IgG, IgM, or IgA antibodies. Furthermore, the diagnostic usefulness of some recombinant antigens in IgG avidity assays have been estimated. Many studies have shown that the nonstandardized whole-cell lysates of *T. gondii* used as antigens for the recognition of specific antibodies in diagnostic tests may be replaced in the future with single recombinant proteins or their various mixtures or chimeric antigens. However, an analysis of the results reported in those publications has shown that different cloning strategies and variations in the recombinant protein purification methods resulted in different levels of sensitivity and specificity being obtained in the diagnostic tests conducted in various laboratories working with the same antigens, for example, the results obtained by Holec et al. (36) and Pfrepper et al. (45) for a recombinant MAG1 antigen. Furthermore, it is difficult to compare the results of some of the studies relating to the application of the same recombinant antigens in the serodiagnosis of toxoplasmosis because the criteria for acute and chronic infection vary among researchers. This should therefore be taken into account when interpreting the results of various studies.

A large number of different recombinant antigens have been produced in the *Escherichia coli* expression system. However, it is known that recombinant proteins produced in *E. coli* often loses their antigenic value due to incorrect folding. Moreover, one of the difficulties in using these recombinant antigens for serodiagnosis in humans is that of *E. coli* antigen contamination in the partially purified recombinant protein preparation. One solution to these problems is the production of new recombinant proteins in eukaryotic expression systems which possesses the entire post-translational modification mechanism and allow the production of recombinant proteins with a conformation that is almost identical to that of the native proteins. In addition, these proteins do not contain contaminants derived from the bacterial cells and, therefore, do not give cross-reactions with human sera. In recent years, the *Pichia* expression system in particular has been used successfully to produced numerous *T. gondii* recombinant antigens (22, 24, 75, 80–83). Thus, there is a need to obtain the new recombinant antigens in different eukaryotic expression systems.

One problem in the field of serologic diagnosis of toxoplasmosis is the lack of a unique and easy-to-use reference test. The reference Sabin-Feldman dye test (84) is performed in only a few clinical laboratories worldwide because it not only requires live parasites but is also expensive and difficult to standardize. Consequently, the development of a standard test based on a combination of recombinant antigens or chimeric proteins remains a major goal for future research work in this area. Moreover, extensive screening of recombinant antigen panels that are representative of the genetic diversity of *Toxoplasma* strains should be undertaken. The search for new, more-effective antigenic proteins of the parasite that can be used as diagnostic tools in the future is also important. Bioinformatics have become very helpful in this, being widely used to predict protein structures, functions, and other biological characteristics (85). Furthermore, the application of recombinant antigenic proteins in other diagnostic assays, such as the rapid lateral flow test based on the immunochromatographic method (86) or the latex agglutination test (87) currently used for the detection of *T. gondii* infection in animals, provides new diagnostic opportunities for researchers.

Certainly, the use of recombinant proteins in the serodiagnosis of toxoplasmosis would be highly beneficial in improving standardization of the tests and reducing their production costs. Furthermore, combining several recombinant antigens that present multiple different epitopes as either a mixture or a chimeric product strongly increases the probability of detecting *T. gondii* antibodies during different stages of the infection. The discovery of markers of new infections and the development of new diagnostic assays which can be used to confirm the acute phase of toxoplasmosis, especially in pregnant women, is also required. Nevertheless, although the diagnostic tests discussed in this article are promising, further work is needed before an immunoassay with recombinant products will be available for clinical purposes.

REFERENCES

1. **Tenter AM, Heckeroth AR, Weiss LM.** 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. **30:**1217–1258.
2. **Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R.** 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet **353:**1829–1833.
3. **Montoya JG.** 2002. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J. Infect. Dis. **185:**S73–S82.
4. **Suzuki LA, Rocha RJ, Rossi CL.** 2001. Evaluation of serological markers for the immunodiagnosis of acute acquired toxoplasmosis. J. Med. Microbiol. **50:**62–70.
5. **Liesenfeld O, Montoya JG, Tathineni NJ, Davis M, Brown BW Jr, Cobb KL, Parsonnet J, Remington JS.** 2001. Confirmatory serologic testing for acute toxoplasmosis and rate of induced abortions among women reported to have positive *Toxoplasma* immunoglobulin M antibody titers. Am. J. Obstet. Gynecol. **184:**140–145.
6. **Liesenfeld O, Press C, Montoya JG, Gill R, Isaac-Renton JL, Hedman K, Remington JS.** 1997. False-positive results in immunoglobulin M (IgM) *Toxoplasma* antibody tests and importance of confirmatory testing: the Platelia Toxo IgM test. J. Clin. Microbiol. **35:**174–178.
7. **Wilson M, Remington JS, Clavet C, Varney G, Press C, Ware D.** 1997. Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. **35:**3112–3115.
8. **Remington JS, Thulliez P, Montoya JG.** 2004. Recent developments for diagnosis of toxoplasmosis. J. Clin. Microbiol. **42:**941–945.
9. **Burg JL, Perelman D, Kasper LH, Ware PL, Boothroyd JC.** 1988. Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii*. J. Immunol. **141:**3584–3591.
10. **Chen XG, Gong Y, Hua-Li Lun ZR, Fung MC.** 2001. High-level expression and purification of immunogenic recombinant SAG1 (P30) of *Toxoplasma gondii* in *Escherichia coli*. Protein Expr. Purif. **23:**33–37.
11. **Hiszczyńska-Sawicka E, Brillowska-Dąbrowska A, Dąbrowski S, Pietkiewicz H, Myjak P, Kur J.** 2003. High yield expression and single-step purification of *Toxoplasma gondii* SAG1, GRA1, and GRA7 antigens in *Escherichia coli*. Protein Exp. Purif. **27:**150–157.
12. **Kotresha D, Poonam D, Muhammad Hafiznur Y, Saadatnia G, Nurulhasanah O, Sabariah O, Tan SY, Izzati Zahidah AK, Rahmah N.** 2012. Recombinant proteins from new constructs of SAG1 and GRA7 sequences and their usefulness to detect acute toxoplasmosis. Trop. Biomed. **29:**129–137.
13. **Prince JB, Auer KL, Huskinson J, Parmley SF, Araujo FG, Remington JS.** 1990. Cloning, expression, and cDNA sequence of surface antigen P22 from *Toxoplasma gondii*. Mol. Biochem. Parasitol. **43:**97–106.
14. **Parmley SF, Sgarlato GD, Mark J, Prince JB, Remington JS.** 1992. Expression, characterization, and serologic reactivity of recombinant surface antigen P22 of *Toxoplasma gondii*. J. Clin. Microbiol. **30:**1127–1133.
15. **Hiszczyńska-Sawicka E, Kur J, Pietkiewicz H, Holec L, Gąsior A, Myjak P.** 2005. Efficient production of the *Toxoplasma gondii* GRA6, p35 and SAG2 recombinant antigens and their applications in the serodiagnosis of toxoplasmosis. Acta Parasitol. **50:**249–254.
16. **Khanaliha K, Motazedian M, Sarkari B, Bandehpour M, Sharifnia Z, Kazemi B.** 2012. Expression and purification of P43 *Toxoplasma gondii* surface antigen. Iran. J. Parasitol. **7:**48–53.
17. **Cesbron-Delauw MF, Guy B, Torpier G, Pierce RJ, Lenzen G, Cesbron JY, Charif H, Lepage P, Darcy F, Lecocq JP, Capron A.** 1989. Molecular characterization of a 23-kilodalton major antigen secreted by *Toxoplasma gondii*. Proc. Natl. Acad. Sci. U. S. A. **86:**7537–7541.
18. **Prince JB, Araujo FG, Remington JS, Burg JL, Boothroyd JC, Sharma SD.** 1989. Cloning of cDNAs encoding a 28 kDa antigen of *Toxoplasma gondii*. Mol. Biochem. Parasitol. **34:**3–14.
19. **Murray A, Mercier C, Decoster A, Lecordier L, Capron A, Cesbron-Delauw MF.** 1993. Muliple B-cell epitopes in a recombinant GRA2 secreted antigen of *Toxoplasma gondii*. Appl. Parasitol. **34:**235–244.
20. **Holec-Gąsior L, Kur J, Hiszczynska-Sawicka E.** 2009. GRA2 and ROP1 recombinant antigens as potential markers for detection of *Toxoplasma gondii*-specific immunoglobulin G in human with acute toxoplasmosis. Clin. Vaccine Immunol. **16:**510–514.
21. **Ching XT, Lau YL, Fong MY, Nissapatorn V.** 2013. Evaluation of *Toxoplasma gondii*-recombinant dense granular protein (GRA2) for serodiagnosis by Western blot. Parasitol. Res. **112:**1229–1236.
22. **Lau YL, Fong MY, Idris MM, Ching XT.** 2012. Cloning and expression of *Toxoplasma gondii* dense granule antigen 2 (GRA2) gene by *Pichia pastoris*. Southeast Asian J. Trop. Med. Public Health **43:**10–16.
23. **Mévélec MN, Chardès T, Mercereau-Puijalon O, Bourguin I, Achbarou A, Dubremetz JF, Bout D.** 1992. Molecular cloning of GRA4, a *Toxoplasma gondii* dense granule protein, recognized by mucosal IgA antibodies. Mol. Biochem. Parasitol. **56:**227–238.
24. **Lau YL, Hasan MT, Thiruvengadam G, Idris MM, Init I.** 2010. Cloning and expression of *Toxoplasma gondii* dense granular protein 4 (GRA4) in *Pichia pastoris*. Trop. Biomed. **27:**525–533.
25. **Holec-Gąsior L, Kur J.** 2010. *Toxoplasma gondii*: Recombinant GRA5 antigen for detection of immunoglobulin G antibodies using enzyme-linked immunosorbent assay. Exp. Parasitol. **124:**272–279.
26. **Lecordier L, Moleon-Borodowsky I, Dubremetz JF, Tourvieille B, Mercier C, Deslee D, Capron A, Cesbron-Delauw MF.** 1995. Characterization of a dense granule antigen of *Toxoplasma gondii* (GRA6) associated to the network of the parasitophorous vacuole. Mol. Biochem. Parasitol. **70:**85–94.
27. **Redlich A, Müller WA.** 1998. Serodiagnosis of acute toxoplasmosis using a recombinant form of the dense granule antigen GRA6 in an enzyme-linked immunosorbent assay. Parasitol. Res. **84:**700–706.
28. **Bonhomme A, Maine GT, Beorchia A, Burlet H, Aubert D, Villena I, Hunt J, Chovan L, Howard L, Brojanac S, Sheu M, Tyner J, Pluot M, Pinon JM.** 1998. Quantitative immunolocalization of a P29 protein (GRA7), a new antigen of *Toxoplasma gondii*. J. Histochem. Cytochem. **46:**1411–1422.
29. **Fischer HG, Stachelhaus S, Sahm M, Meyer HE, Reichmann G.** 1998. GRA7, an excretory 29 kDa *Toxoplasma gondii* dense granule antigen released by infected host cells. Mol. Biochem. Parasitol. **91:**251–262.
30. **Jacobs D, Dubremetz JF, Loyens A, Bosman F, Saman E.** 1998. Identification and heterologous expression of a new dense granule protein (GRA7) from *Toxoplasma gondii*. Mol. Biochem. Parasitol. **91:**237–249.
31. **Selseleh M, Keshavarz H, Mohebali M, Shojaee S, Selseleh M, Eshragian MR, Mansouri F, Modarressi MH.** 2012. Production and evaluation of *Toxoplasma gondii* recombinant GRA7 for serodiagnosis of human infections. Korean J. Parasitol. **50:**233–238.
32. **Saavedra R, De Meuter F, Decourt JL, Hérion P.** 1991. Human T cell clone identifies a potentially protective 54-kDa protein antigen of *Toxoplasma gondii* cloned and expressed in *Escherichia coli*. J. Immunol. **147:**1975–1982.
33. **van Gelder V, Bosman F, De Meuter F, Van Heuverswyn H, Hérion P.** 1993. Serodiagnosis of toxoplasmosis by using a recombinant form of the 54-kilodalton rhoptry antigen expressed in *Escherichia coli*. J. Clin. Microbiol. **31:**9–15.
34. **Martin V, Arcavi M, Santillan G, Amendoeira MRR, De Souza Neves E, Griemberg G, Guarnera E, Garberi JC, Angel SO.** 1998. Detection of human *Toxoplasma*-specific immunoglobulins A, M, and G with a recombinant *Toxoplasma gondii* rop2 protein. Clin. Diagn. Lab. Immunol. **5:**627–631.
35. **Nockemann SH, Długońska H, Henrich B, Kitzerow A, Daubener W.** 1998. Expression, characterization and serological reactivity of a 41 kDa excreted-secreted antigen (ESA) from *Toxoplasma gondii*. Mol. Biochem. Parasitol. **97:**109–121.
36. **Holec L, Hiszczyńska-Sawicka E, Gąsior A, Brillowska-Dąbrowska A, Kur J.** 2007. Use of MAG1 recombinant antigen for detection of *Toxoplasma gondii* infection in humans. Clin. Vaccine Immunol. **14:**220–225.
37. **Holec L, Gąsior A, Brillowska-Dąbrowska A, Kur J.** 2008. *Toxoplasma gondii*: enzyme-linked immunosorbent assay using different fragments of recombinant microneme protein (MIC1) for detection of immunoglobulin G antibodies. Exp. Parasitol. **119:**1–6.
38. **Kotresha D, Noordin R.** 2010. Recombinant proteins in the diagnosis of toxoplasmosis. APMIS **118:**529–542.
39. **Harning D, Spenter J, Metsis A, Vuust J, Petersen E.** 1996. Recombinant *Toxoplasma gondii* surface antigen 1 (P30) expressed in *Escherichia coli* is recognized by human *Toxoplasma*-specific immunoglobulin M (IgM) and IgG antibodies. Clin. Diagn. Lab. Immunol. **3:**355–357.
40. **Jacobs D, Vercammen M, Saman E.** 1999. Evaluation of recombinant dense granule antigen 7 (GRA7) of *Toxoplasma gondii* for detection of immunoglobulin G antibodies and analysis of a major antigenic domain. Clin. Diagn. Lab. Immunol. **6:**24–29.
41. **Aubert D, Maine GT, Villena I, Hunt JC, Howard L, Sheu M, Brojanac S, Chovan LE, Nowlan SF, Pinon JM.** 2000. Recombinant antigens to detect *Toxoplasma gondii*-specific immunoglobulin G and immunoglob-

ulin M in human sera by enzyme immunoassay. J. Clin. Microbiol. **38:** 1144–1150.
42. **Lecordier L, Fourmaux MP, Mercier C, Dehecq E, Masy E, Cesbron-Delauw MF.** 2000. Enzyme-linked immunosorbent assay using recombinant dense granule antigens GRA6 and GRA1 of *Toxoplasma gondii* for detection of immunoglobulin G antibodies. Clin. Diagn. Lab. Immunol. **7:**607–611.
43. **Pietkiewicz H, Hiszczyńska-Sawicka E, Kur J, Petersen E, Nielsen HV, Stankiewicz M, Andrzejewska I, Myjak P.** 2004. Usefulness of *Toxoplasma gondii*-specific recombinant antigens in serodiagnosis of human toxoplasmosis. J. Clin. Microbiol. **42:**1779–1781.
44. **Buffolano W, Beghetto E, Del Pezzo M, Spadoni A, Di Cristina M, Petersen E, Gargano N.** 2005. Use of recombinant antigens for early postnatal diagnosis of congenital toxoplasmosis. J. Clin. Microbiol. **43:** 5916–5924.
45. **Pfrepper KI, Enders G, Gohl M, Krczal D, Hlobil H, Wassenberg D, Soutschek E.** 2005. Seroreactivity to and avidity for recombinant antigens in toxoplasmosis. Clin. Diagn. Lab. Immunol. **12:**977–982.
46. **Beghetto E, Spadoni A, Bruno L, Buffolano W, Gargano N.** 2006. Chimeric antigens of *Toxoplasma gondii*: toward standardization of toxoplasmosis serodiagnosis using recombinant products. J. Clin. Microbiol. **44:**2133–2140.
47. **Lau YL, Fong MY.** 2008. *Toxoplasma gondii*: serological characterization and immunogenicity of recombinant surface antigen 2 (SAG2) expressed in the yeast *Pichia pastoris*. Exp. Parasitol. **119:**373–378.
48. **Wu K, Chen XG, Li H, Yan H, Yang PL, Lun ZR, Zhu XQ.** 2009. Diagnosis of human toxoplasmosis by using the recombinant truncated surface antigen 1 of *Toxoplasma gondii*. Diagn. Microbiol. Infect. Dis. **64:**261–266.
49. **Suzuki Y, Ramirez R, Press C, Li S, Parmley S, Thulliez P, Remington JS.** 2000. Detection of immunoglobulin M antibodies to P35 antigen of *Toxoplasma gondii* for serodiagnosis of recently acquired infection in pregnant women. J. Clin. Microbiol. **38:**3967–3970.
50. **Li S, Maine G, Suzuki Y, Araujo FG, Galvan G, Remington JS, Parmley S.** 2000. Serodiagnosis of recently acquired *Toxoplasma gondii* infection with a recombinant antigen. J. Clin. Microbiol. **38:**179–184.
51. **Nigro M, Gutierrez A, Hoffer AM, Clemente M, Kaufer F, Carral L, Martin V, Guarnera EA, Angel SO.** 2003. Evaluation of *Toxoplasma gondii* recombinant proteins for the diagnosis of recently acquired toxoplasmosis by an immunoglobulin G analysis. Diagn. Microbiol. Infect. Dis. **47:**609–613.
52. **Beghetto E, Buffolano W, Spadoni A, Del Pezzo M, Di Cristina M, Minenkova O, Petersen E, Felici F, Gargano N.** 2003. Use of an immunoglobulin G avidity assay based on recombinant antigens for diagnosis of primary *Toxoplasma gondii* infection during pregnancy. J. Clin. Microbiol. **41:**5414–5418.
53. **Golkar M, Rafati S, Abdel-Latif MS, Brenier-Pinchart MP, Fricker-Hidalgo H, Sima BK, Babaie J, Pelloux H, Cesbron-Delauw MF, Mercier C.** 2007. The dense granule protein GRA2, a new marker for the serodiagnosis of acute *Toxoplasma* infection: comparison of sera collected in both France and Iran from pregnant women. Diagn. Microbiol. Infect. Dis. **58:**419–426.
54. **Béla SR, Oliveira Silva DA, Cunha-Júnior Pirovani JPCP, Chaves-Borges FA, Reis de Carvalho F, Carrijo de Oliveira T, Mineo JR.** 2008. Use of SAG2A recombinant *Toxoplasma gondii* surface antigen as a diagnostic marker for human acute toxoplasmosis: analysis of titers and avidity of IgG and IgG1 antibodies. Diagn. Microbiol. Infect. Dis. **62:**245–254.
55. **Golkar M, Azadmanesh K, Khalili G, Khoshkholgh-Sima B, Babaie J, Mercier C, Brenier-Pinchart MP, Fricker-Hidalgo H, Pelloux H, Cesbron-Delauw MF.** 2008. Serodiagnosis of recently acquired *Toxoplasma gondii* infection in pregnant women using enzyme-linked immunosorbent assays with a recombinant dense granule GRA6 protein. Diagn. Microbiol. Infect. Dis. **61:**31–39.
56. **Lappalainen M, Koskela P, Koskiniemi M, Ammälä P, Hiilesmaa V, Teramo K, Raivio KO, Remington JS, Hedman K.** 1993. Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. J. Infect. Dis. **167:**691–697.
57. **Jenum PA, Stray-Pedersen B, Gundersen AG.** 1997. Improved diagnosis of primary Toxoplasma gondii infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J. Clin. Microbiol. **35:**1972–1977.
58. **Liesenfeld O, Montoya JG, Kinney S, Press C, Remington JS.** 2001. Effect of testing for IgG avidity in the diagnosis of *Toxoplasma gondii* infection in pregnant women: experience in a US reference laboratory. J. Infect. Dis. **183:**1248–1253.
59. **Montoya JG, Liesenfeld O, Kinney S, Press C, Remington JS.** 2002. VIDAS test for avidity of *Toxoplasma*-specific immunoglobulin G for confirmatory testing of pregnant women. J. Clin. Microbiol. **40:**2504–2508.
60. **Marcolino PT, Silva DA, Leser PG, Camargo ME, Mineo JR.** 2000. Molecular markers in acute and chronic phases of human toxoplasmosis: determination of immunoglobulin G avidity by Western blotting. Clin. Diagn. Lab. Immunol. **7:**384–389.
61. **Sickinger E, Gay-Andrieu F, Jonas G, Schultess J, Stieler M, Smith D, Hausmann M, Stricker R, Stricker R, Dhein J, Braun HB.** 2008. Performance characteristics of the new ARCHITECT Toxo IgG and Toxo IgG Avidity assays. Diagn. Microbiol. Infect. Dis. **62:**235–244.
62. **Holec-Gąsior L, Drapała D, Lautenbach D, Kur J.** 2010. *Toxoplasma gondii:* usefulness of ROP1 recombinant antigen in an immunoglobulin G avidity assay for diagnosis of acute toxoplasmosis in humans. Pol. J. Microbiol. **59:**307–310.
63. **Elyasi H, Babaie J, Fricker-Hidalgo H, Brenier-Pinchart MP, Zare M, Sadeghiani G, Assmar M, Pelloux H, Golkar M.** 2010. Use of dense granule antigen GRA6 in an immunoglobulin G avidity test to exclude acute *Toxoplasma gondii* infection during pregnancy. Clin. Vaccine Immunol. **17:**1349–1355.
64. **Pietkiewicz H, Hiszczyńska-Sawicka E, Kur J, Petersen E, Nielsen HV, Paul M, Stankiewicz M, Myjak P.** 2007. Usefulness of *Toxoplasma gondii* recombinant antigens (GRA1, GRA7, SAG1) in an immunoglobulin G avidity test for serodiagnosis of toxoplasmosis. Parasitol. Res. **100:**333–337.
65. **Cozon GJ, Ferrandiz J, Nebhi H, Wallon M, Peyron F.** 1998. Estimation of the avidity of immunoglobulin G for routine diagnosis of chronic *Toxoplasma gondii* infection in pregnant women. Eur. J. Clin. Microbiol. Infect. Dis. **17:**32–36.
66. **Pelloux H, Brun E, Vernet G, Marcillat S, Jolivet M, Guergour D, Hidalgo H, Fleuret A, Ambroise-Thomas P.** 1998. Determination of anti-*Toxoplasma gondii* immunoglobulin G avidity: adaptation to the VIDAS System (BioMerieux). Diagn. Microbiol. Infect. Dis. **32:**69–73.
67. **Johnson AM, Roberts H, Tenter AM.** 1992. Evaluation of a recombinant antigen ELISA for the diagnosis of acute toxoplasmosis and comparison with traditional antigen ELISAs. J. Med. Microbiol. **37:**404–409.
68. **Tenter AM, Johnson AM.** 1991. Recognition of recombinant *Toxoplasma gondii* antigens by human sera in an ELISA. Parasitol. Res. **77:**197–203.
69. **Li S, Galvan G, Araujo FG, Suzuki Y, Remington JS, Parmley S.** 2000. Serodiagnosis of recently acquired *Toxoplasma gondii* infection using an enzyme-linked immunosorbent assay with a combination of recombinant antigens. Clin. Diagn. Lab. Immunol. **7:**781–787.
70. **Roggen EL.** 2006. Recent developments with B-cell epitope identification for predictive studies. J. Immunotoxicol. **3:**137–149.
71. **Maksimov P, Zerweck J, Maksimov A, Hotop A, Gross U, Pleyer U, Spekker K, Däubener W, Werdermann S, Niederstrasser O, Petri E, Mertens M, Ulrich RG, Conraths FJ, Schares G.** 2012. Peptide microarray analysis of *in silico*-predicted epitopes for serological diagnosis of *Toxoplasma gondii* infection in humans. Clin. Vaccine Immunol. **19:**865–874.
72. **Cardona N, de la Torre A, Siachoque H, Patarroyo MA, Gomez-Marin JE.** 2009. *Toxoplasma gondii*: P30 peptides recognition pattern in human toxoplasmosis. Exp. Parasitol. **123:**199–202.
73. **Mévélec MN, Mercereau-Puijalon O, Buzoni-Gatel D, Bourguin I, Chardès T, Dubremetz JF, Bout D.** 1998. Mapping of B epitopes in GRA4, a dense granule antigen of *Toxoplasma gondii* and protection studies using recombinant proteins administered by the oral route. Parasite Immunol. **20:**183–195.
74. **Reineke U.** 2009. Antibody epitope mapping using *de novo* generated synthetic peptide libraries. Methods Mol. Biol. **524:**203–211.
75. **Lau YL, Thiruvengadam G, Lee WW, Fong MF.** 2011. Immunogenic characterization of the chimeric surface antigen 1 and 2 (SAG1/2) of *Toxoplasma gondii* expressed in the yeast *Pichia pastoris*. Parasitol. Res. **109:** 871–878.
76. **Holec-Gąsior L, Ferra B, Drapała D, Lautenbach D, Kur J.** 2012. A new MIC1-MAG1 recombinant chimeric antigen can be used instead of the *Toxoplasma gondii* lysate antigen in serodiagnosis of human toxoplasmosis. Clin. Vaccine Immunol. **19:**57–63.
77. **Holec-Gąsior L, Ferra B, Drapała D.** 2012. MIC1-MAG1-SAG1 chimeric protein, a most effective antigen for detection of human toxoplasmosis. Clin. Vaccine Immunol. **19:**1977–1979.
78. **Dai J, Jiang M, Wang Y, Qu L, Gong R, Si J.** 2012. Evaluation of a

recombinant multiepitope peptide for serodiagnosis of *Toxoplasma gondii* infection. Clin. Vaccine Immunol. **19:**338–342.
79. **Dai JF, Jiang M, Qu LL, Sun L, Wang YY, Gong LL, Gong RJ, Si J.** 2013. *Toxoplasma gondii*: enzyme-linked immunosorbent assay based on a recombinant multi-epitope peptide for distinguishing recent from past infection in human sera. Exp. Parasitol. **133:**95–100.
80. **Biemans R, Grégoire D, Haumont M, Bosseloir A, Garcia L, Jacquet A, Dubeaux C, Bollen A.** 1998. The conformation of purified *Toxoplasma gondii* SAG1 antigen, secreted from engineered *Pichia pastoris*, is adequate for serorecognition and cell proliferation. J. Biotechnol. **66:**137–146.
81. **Chang PY, Fong MY, Nissapatorn V, Lau YL.** 2011. Evaluation of *Pichia pastoris*-expressed recombinant rhoptry protein 2 of *Toxoplasma gondii* for its application in diagnosis of toxoplasmosis. Am. J. Trop. Med. Hyg. **85:**485–489.
82. **Thiruvengadam G, Init I, Fong MY, Lau YL.** 2011. Optimization of the expression of surface antigen SAG1/2 of *Toxoplasma gondii* in the yeast *Pichia pastoris*. Trop. Biomed. **28:**506–513.
83. **Zhou H, Gu Q, Zhao Q, Zhang J, Cong H, Li Y, He S.** 2007. *Toxoplasma gondii*: expression and characterization of a recombinant protein containing SAG1 and GRA2 in *Pichia pastoris*. Parasitol. Res. **100:**829–835.
84. **Sabin AB, Feldman HA.** 1948. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (*Toxoplasma*). Science **108:**660–663.
85. **Bai Y, He S, Zhao G, Chen L, Shi N, Zhou H, Cong H, Zhao Q, Zhu XQ.** 2012. *Toxoplasma gondii*: bioinformatics analysis, cloning and expression of a novel protein TgIMP1. Exp. Parasitol. **132:**458–464.
86. **Huang X, Xuan X, Hirata H, Yokoyama N, Xu L, Suzuki N, Igarashi I.** 2004. Rapid immunochromatographic test using recombinant SAG2 for detection of antibodies against *Toxoplasma gondii* in cats. J. Clin. Microbiol. **42:**351–353.
87. **Jiang T, Gong D, Ma LA, Nie H, Zhou Y, Yao B, Zhao J.** 2008. Evaluation of a recombinant MIC3 based latex agglutination test for the rapid serodiagnosis of *Toxoplasma gondii* infection in swines. Vet. Parasitol. **158:**51–56.

Lucyna Holec-Gąsior is an Assistant Professor at the Department of Microbiology, Gdansk University of Technology, Poland, where she is a member of the Molecular Diagnostic research group. She graduated in biotechnology, with a molecular biotechnology major, from the Faculty of Chemistry, Gdansk University of Technology, in 2003 and received a Ph.D. in chemical sciences in biotechnology from the same university in 2007. Her current research interests are focused on the problem of toxoplasmosis diagnosis, with new diagnostic tools useful for the detection of specific anti-*T. gondii* antibodies in serum samples from humans and different domestic animals being the subject of her special interest. As a member of the scientific team at the Gdansk University of Technology's Department of Microbiology, she has been leading research related to obtaining new diagnostic kits for several years now.