Research Article

Detection of immunogenic parasite and host-specific proteins in the sera of active and chronic individuals infected with *Toxoplasma gondii*

*Toxoplasma gondii* infection in pregnant women may result in abortion and foetal abnormalities, and may be life-threatening in immunocompromised hosts. To identify the potential infection markers of this disease, 2-DE and Western blot methods were employed to study the parasite circulating antigens and host-specific proteins in the sera of *T. gondii*-infected individuals. The comparisons were made between serum protein profiles of infected (*n* = 31) and normal (*n* = 10) subjects. Antigenic proteins were identified by immunoblotting using pooled sera and monoclonal anti-human IgM-HRP. Selected protein spots were characterised using mass spectrometry. Prominent differences were observed when serum samples of *T. gondii*-infected individuals and normal controls were compared. A significant up-regulation of host-specific proteins, *α*₂- HS glycoprotein and *α*₁-B glycoprotein, was also observed in the silver-stained gels of both active and chronic infections. However, only *α*₂-HS glycoprotein and *α*₁-B glycoprotein in the active infection showed immunoreactivity in Western blots. In addition, three spots of *T. gondii* proteins were detected, namely (i) hypothetical protein chrXII: 3984434-3 TGME 49, (ii) dual specificity protein phosphatase, catalytic domain TGME 49 and (iii) NADPH-cytochrome p450 reductase TGME 49. Thus, 2-DE approach followed by Western blotting has enabled the identification of five potential infection markers for the diagnosis of toxoplasmosis: three are parasite-specific proteins and two are host-specific proteins.

**Keywords:**
2-DE / Host-specific protein / Immunoblot / Parasite protein / *Toxoplasma gondii*

DOI 10.1002/elps.201000038

1 Introduction

Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*, an intracellular protozoan parasite. Approximately one-third of the world’s population may have been exposed to the parasite [1]. More than 60 million and 16–40% of people in the United States and United Kingdom, respectively, are estimated to be infected with *T. gondii*, whereas in continental Europe, the infection estimates reached up to 50–80% [1–4]. Although the course of the infection is generally mild and sometimes symptomless, it can cause significant morbidity and mortality in the developing foetuses and immunocompromised individuals. In recent years, the importance of toxoplasmosis has been increasingly recognised due to the increasing number of the latter group of individuals. Recognition of an active infection is thus important for enabling clinicians to commence treatment. Although chronic infection does not require treatment, it may however revert to an active infection in immunocompromised individuals. Therefore, the availability of a method to detect the parasite antigen in human serum samples would be desirable [5, 6].

Serum is a very accessible sample and likely able to show the numerous events taking place *in vivo* as a systemic mirror. From the serum proteome, besides the potential of identifying circulating parasite antigens, information on functional cellular processes in human host system may also be elucidated. Direct detection of antigenic infection markers of *T. gondii* antigen in patients’ sera has not been reported. Recently, 2-DE in combination with mass spectrometry and immunoblot on serum proteome has been successfully used to study infections caused by several organisms namely hepatitis B virus, hepatitis C virus (HCV), dengue virus and *Helicobacter pylori* [7–11].

**Correspondence:** Dr. Rahmah Noordin, Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Penang, Malaysia

E-mail: rahmah8485@gmail.com
Fax: +604-6534803

**Abbreviations:** ABG, *α*₁-B glycoprotein; AHS, *α*₂-HS glycoprotein; CA, circulating antigen

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

www.electrophoresis-journal.com
However, the focus of these studies was mainly on the basic molecular and cellular proteomic functions and was not directed at studying proteins that may be potential targets for disease diagnosis.

In an effort to increase the sensitivity and the specificity of the diagnosis of active toxoplasmosis, we investigated new infection markers that individually or in combination with other markers may potentially be used in the future for the development of diagnostic test(s). The approach undertaken was by performing 2-DE followed by Western blotting to compare the profiles of sera from infected individuals with normal healthy sera, followed by characterisation of potential infection markers using mass spectrometry.

2 Materials and methods

2.1 Samples for analysis

In a previous study, we have developed an ELISA for the detection of Toxoplasma circulating antigen (CA) [12]. In this assay, monoclonal anti-Toxoplasma SAG1 (p30) was used as the capture antibody to detect T. gondii CAs in patients’ serum samples. Subsequently, rabbit anti-Toxoplasma IgG was added, followed by peroxidase goat anti-rabbit IgG and finally 2,2-azino-di-[3-ethylbenzthiazoline sulphonate] was used as the substrate. The same serum samples were also used in the present study, which was collected according to the guidelines of USM Human Research Ethics Committee. The samples were categorised as follows: (i) category 1: active toxoplasmosis; (ii) category 2: chronic toxoplasmosis; and (iii) category 3: normal serum (i.e. with active toxoplasmosis), preincubated with RF absorbent (Serion, Germany), at a dilution of 1:100. After a washing step, it was incubated with monoclonal anti-human IgM-HRP (Invitrogen, USA) at 1:3000 dilution for 1 h at room temperature. After another washing step, the membrane was processed for visualisation using chemiluminescence substrate on 18 cm × 24 cm films (Sigma, USA).

2.4 Western blotting

The pooled serum samples (category 1, 2 or 3) from ready ran 2-D gels were transferred onto nitrocellulose membrane using the Multiphor II Novablot semi-dry system (Pharmacia, Sweden). The blotted nitrocellulose membrane was blocked and washed 3 × with TBS–Tween-20. It was then incubated overnight (4°C) with pooled sera from category 1 samples (i.e. with active toxoplasmosis), preincubated with RF absorbent, at a dilution of 1:100. After a washing step, it was incubated with monoclonal anti-human IgM-HRP (Invitrogen, USA) at 1:3000 dilution for 1 h at room temperature. After another washing step, the membrane was processed for visualisation using chemiluminescence substrate on 18 cm × 24 cm films (Sigma, USA).

2.5 Differential image analysis

The LabScan image scanner (Version 5; Amersham, Germany) was used to capture and store images of 2-DE gels. The ImageMaster™ 2-D Platinum Software (Version 5; Amersham) was used to evaluate the protein profiles and present the information obtained from the 2-DE gels. To detect the proteins that were differentially expressed in sera, the percentages of volume contributions (vol%) were calculated. This refers to the volume percentage of a protein taken against the total spot volume of all proteins including the unresolved peptides in each gel. Data obtained in such expressions are independent of variations attributed to protein loading and staining.

2.6 Statistical analysis

Levels of proteins in the gels are presented as mean vol% ± SD. The variance ratio test (F) was used to analyse the differences between control subjects and T. gondii-infected individuals and to examine the correlation between the variables. A p-value of < 0.05 was considered statistically significant.

2.7 Mass spectrometry analysis and database search

Selected spots were excised and subjected to in-gel tryptic digestion by using a commercial in-gel tryptic digestion kit (Agilent, USA). The mass spectrometry analysis and database search were performed at the Proteomic Centre, Faculty of Biological Sciences, National University of Singapore. The peptide digest from each gel spot was mixed with 1.2 μL of CHCA matrix solution (5 mg/mL of
cyano-4-hydroxy-cinnamic acid in 0.1% TFA and 50% ACN) and spotted onto a MALDI target plate. The peptide mass spectra were obtained using the ABI 4800 Proteomics Analyzer MALDI-TOF/TOF Mass Spectrometer (Applied Biosystems, Foster City, CA, USA). For MS analysis, typically 1000 shots were accumulated for each sample. MS data were automatically obtained with the five most intense ions selected for MS/MS. The peptides were subsequently subjected to MS/MS analyses using air with a collision energy of 2 kV and a collision gas pressure of $\sim 1 \times 10^{-6}$ Torr. The stop conditions were implemented so that 2000–3000 shots were accumulated depending on the quality of the spectra. MASCOT search engine (version 2.1; Matrix Science, London, UK) was used to search all the tandem mass spectra.

GPS Explorer\textsuperscript{TM} software Version 3.6 (Applied Biosystems) was used to create and search files with MASCOT search engine for peptide and protein identification. The search parameters allowed for N-terminal acetylation, cysteine C-terminal carbamidomethylation and methionine oxidation. The peptide mass tolerance and the fragment mass tolerance were set to 150 ppm and $\pm 0.4$ Da, respectively.

Proteins were initially identified by comparing their peptide masses with the built-in search engine ProteinPilot proteomics software (4800) Proteomics Analyzer (Applied Biosystems), a computer-generated database of tryptic peptides from known proteins. The score, which reflects the match of theoretically and experimentally determined masses, was calculated and assigned. Analyses were conducted using the International Protein Index human database (http://www.ebi.ac.uk/IPI/) NCBI, Unigene human databases and ToxoDB database (http://toxodb.org/common/downloads/release-5.2/Tgondii/).

### 3 Results

#### 3.1 Serum protein profiles

The separation of unfractionated sera from normal controls by 2-DE generated high-resolution profiles comprising resolved clusters of proteins including albumin, and the heavy and light chains of IgA, IgG and IgM. Other proteins observed in normal sera were $\alpha_2$-HS glycoprotein (AHS), $\alpha_2$-B glycoprotein (ABG), $\alpha_2$-antitrypsin, haptoglobin $\beta$ chains and clusterin (CLU2) (Fig. 1A) [13]. In comparison with normal sera, differences in protein profiles of human serum proteins were observed with sera from both active and chronically infected individuals, namely in the expressions of AHS and ABG. Figure 1B and C shows typical 2-DE serum protein profiles of individuals with active and chronic T. gondii infection. The unfractionated serum samples of the patients and normal controls were subjected to 2-DE and silver stained. Protein spots were compared and analysed using ImageMaster\textsuperscript{TM} 2-D Platinum Software Version 5. Besides the five clearly detectable clusters of protein spots, unknown proteins of 29, 33 and 38 kDa were detected only in the serum protein profile of acute $T. gondii$-infected patient.

#### 3.2 Image analysis of 2-DE gels

The clusters of protein spots were analysed by densitometry software. Image analyses were carried out on the five proteins, namely ABG, AHS and three unknown proteins of 29, 33 and 38 kDa (Table 2); and all five were found to be aberrantly expressed in patients’ samples. The expression of AHS and ABG in the sera from infected individuals was significantly different compared to their normal controls. The expression of AHS was significantly up-regulated at 2.38-fold ($p<0.05$) and 1.71-fold ($p<0.05$) in active and chronic toxoplasmosis sera, respectively.

Figure 1. Representative 2-DE serum protein profiles of the (A) normal control and individuals with (B) active and (C) chronic $T. gondii$ infection. The unfractionated serum samples of the patients and normal controls were subjected to 2-DE and silver stained. Protein spots were compared and analysed using ImageMaster\textsuperscript{TM} 2-D Platinum Software Version 5. Besides the five clearly detectable clusters of protein spots, unknown proteins of 29, 33 and 38 kDa were detected only in the serum protein profile of acute $T. gondii$-infected patient.
whereas the expression of ABG was significantly up-regulated at 1.88-fold ($p < 0.05$) for active sera and 1.35-fold for chronic sera ($p < 0.05$) (Table 3). However, there was no significant difference in the expression of AHS and ABG between active and chronic infection sera ($p > 0.05$). In the case of the three unknown antigens of 29, 33 and 38 kDa, their expressions were only observed in the serum samples from active infections (Fig. 1B or Table 1). Of the 21 sera from active infection, 29, 33 and 38 kDa were seen in 19 (90.5%), 14 (66.7%) and 11 (52.4%) of the stained 2-DE gels with the average vol% of 0.08665, 0.04657 and 0.00625, respectively.

### 3.3 Detection of immunogenic proteins by 2-DE immunoblotting

The 2-DE immunoblotting showed no significant difference between the serum profiles of chronic sera as compared to normal controls. The differences were observed between the immunoblots of active toxoplasmosis sera and the immunoblots of both chronic infection sera and normal sera. As shown in Fig. 2A–C, unlike control and chronic toxoplasmosis sera, the blot from active toxoplasmosis sera exhibited several immunogenic host-specific proteins namely ABG, AHS and three unknown antigens of 29, 33 and 38 kDa proteins.

### 3.4 Identification of proteins using mass spectrometry

In this study, only the ABG and the AHS, and the unknown 29, 33 and 38 kDa proteins were further analysed by subjecting the protein spot clusters to MALDI-TOF/TOF analysis, followed by database search (Tables 1 and 2).

Comparisons of the MS/MS spectra and the score of AHS and ABG from the sera of controls and active toxoplasmosis were compared (Table 2). Significant matches, with a search score of more than 82, were obtained for ABG and AHS from both types of sera. However, additional peaks in the spectrum were observed in these two proteins from active toxoplasmosis serum (data not shown); thus, this might indicate the modifications of these proteins in the sample.

The three additional antigenic spots from active toxoplasmosis sera of molecular weights 29, 33 and 38 kDa were also identified as (i) hypothetical protein chrXII: 3984434-3 TGME49, (ii) dual specificity protein phosphatase, catalytic domain TGME49 and (iii) NADPH-cytochrome p450 reductase, putative location = TG

### 4 Discussion

There are many commercially available antibody detection tests for toxoplasmosis; however, they displayed a lot of variability in results, mostly due to the variations in the antigens employed.

In order to develop an antigen detection test, one needs to determine the antigen that circulate in the body fluids of infected patients such as serum/plasma and CSF samples. Although a number of studies have been performed on developing such an assay for *T. gondii*, such a test is not commercially available nor does it form a part of a panel of diagnostic test in reference diagnostic laboratories. This may partly due to the lack of good data regarding *Toxoplasma* antigens that circulate in the blood in active infection. Towards this aim, this study was conducted to characterise *T. gondii* antigens and/or host-specific proteins in serum samples of infected individuals.

---

**Table 1. Mass spectrometric identification of unknown protein spot clusters from serum protein profiles using MASCOT search engine and ToxoDB database**

| Unknown protein spot (kDa) | Spot ID | Accession number | $p_I$ | Theoretical mass (Da) | Sequence coverage (%) | Search scores | Queries match | Expected value |
|---------------------------|---------|------------------|-------|-----------------------|-----------------------|---------------|---------------|----------------|
| 29                        | Toxoplasma gondii_ME49 | product = hypothetical protein chrXII: 3984434-3 TGME49 048560 | 4.64  | 28060                | 24                   | 35          | 8             | 2.80          |
| 33                        | Toxoplasma gondii_ME49 | product = dual specificity protein phosphatase, catalytic domain TGME49 021500 | 4.06  | 110164               | 21                   | 47          | 20            | 0.16          |
| 38                        | Toxoplasma gondii_ME49 | product = NADPH-cytochrome p450 reductase, putative location = TG TGME49 019630 | 6.21  | 104892               | 20                   | 32          | 13            | 4.7           |
The 2-DE proteomic technology has been widely used to identify specific proteins in bacteria or virus for potential vaccine candidates or serodiagnostic targets [7–11]. Thus far, the limited proteomic studies on T. gondii used parasite cell culture or lysate to investigate its pathogenic mechanism and to identify diagnostic marker(s) [16–18]. Recently, Reichmann et al. [19] used 2-DE method to investigate the profile of T. gondii tachyzoites lysate and succeeded in determining the target antigen of a T-cell clone from mouse infected with T. gondii. Nielsen et al. [20] developed the 2-DE immunoblotting assay that allowed early detection and differentiation between passively transferred maternal T. gondii-specific IgG antibodies and antibodies synthesised by the newborn child which may lead to improvement in the diagnosis of congenital toxoplasmosis. However, the above approach will result in the development of antibody detection test, rather than an antigen detection test.

Ma et al. [21] established a complete proteome map of T. gondii soluble tachyzoites antigens. Of the fractionated 1227 protein spots, only 426 spots were verified using Swiss-Prot and NCBI databases. Among these, 230 spots (54%) were attributed to T. gondii. However, the Western blot analysis revealed 30 antigenic spots that reacted with infected rabbit anti T. gondii serum, and out of those, only 16 spots were finally identified as being T. gondii-specific proteins. Their study thus showed the current limitation of the databases and proteomics technologies.

In this study, the ToxoDB data sets were used to search MS/MS peptides against the hypothetical T. gondii proteome (mainly based on ME49 strain), which comprised all computationally predicted and experimental sequences available [22–24]. The database contains protein data from approximately 8000 genes of strain ME49 (http://toxodb.org/common/downloads/release-5.2/Tgondii/). Although ToxoDB contains the most up-to-date annotation on Toxoplasma sequences provided by the sequencing centres, it is not 100% completed; thus, it cannot be ruled out that some genes are missed due to incorrect annotation or are not annotated at all. Experimental verifications are needed to assess whether the data sets offer a comprehensive view of the T. gondii proteome [25]. Thus, the results of this study will help to provide the evidence for some of the predicted sequences.

Studies on T. gondii CA thus far were limited to the study of its detection and identification of the MWs of CAs by SDS–PAGE and Western blot analysis. In one study, sera containing circulating immune complexes from children with congenital toxoplasmosis and from pregnant women with IgM antibodies to Toxoplasma were found to demonstrate 55–58, 48, 44, 38, 30 and 26 kDa antigenic components [26]. In other studies, IgM-positive sera from active toxoplasmosis patients recognised Toxoplasma proteins that were 36 and 60 kDa, whereas a 12-kDa protein was observed in the chronic phase [27,28]. A study by Attallah et al. [6] demonstrated the presence of a highly immunogenic T. gondii antigen of 36 kDa in the sera of women with active toxoplasmosis infection. Apart from human studies, Shojaee et al. [29] described the detection of T. gondii antigens in the sera of experimentally infected mice and found six bands of antigenic components with MWs of 22, 30, 32, 38, 97 and 106 kDa.

In this present study, proteomics approach used separation of patients’ sera (active or chronic infection, presumably containing Toxoplasma antigen) by 2-DE and immunoblotting using anti-Toxoplasma antibodies from pooled active infection

![Figure 2. 2-DE Western blot of the (A) pooled normal control, (B) pooled active toxoplasmosis sera and (C) pooled chronic toxoplasmosis sera, probed with pooled active toxoplasmosis sera and monoclonal anti-human IgM-HRP. Unlike the control and chronic toxoplasmosis sera, the blot from active toxoplasmosis sera exhibited several immunogenic host-specific proteins namely ABG, AHS and three unknown antigens of 29, 33 and 38 kDa proteins.](image-url)
Table 2. Mass spectrometric identification of host-specific protein spot clusters from serum protein profiles using MASCOT search engine and NCBI database

| Spot ID matched | MASCOT accession number | pI/Mr (kDa) range (theoretical) | Theoretical mass | Search score | Expected search | Queries |
|-----------------|-------------------------|---------------------------------|------------------|--------------|----------------|---------|
| AHS (acute toxoplasmosis sera) | gi|2521983 | 4.3–4.5/59–64 | 40 197 | 169 | 1e–010 | 9 |
| AHS (negative control sera) | gi|2521983 | 40 197 | 175 | 2.6e–011 | 10 |
| ABG (acute toxoplasmosis sera) | gi|69990 | 5.0–5.4/77–82 | 52 479 | 204 | 3.2e–014 | 13 |
| ABG (negative control sera) | gi|69990 | 52 479 | 341 | 6.2e–028 | 18 |

Table 3. The relative expression of antigens and host-specific proteins in the sera of toxoplasmosis patients at different stages of infection

| Serum proteins | Statistical analysis (fold changes and significance) | ANOVA |
|----------------|-----------------------------------------------|-------|
|                | Acute toxoplasmosis (n = 21) | Chronic toxoplasmosis (n = 10) |
| AHS            | 2.38-fold, p < 0.05 (SD = 0.740) | 1.71-fold, p < 0.05 (SD = 0.822) | 0.1337 |
| ABG            | 1.88-fold, p < 0.05 (SD = 0.522) | 1.35-fold, p < 0.05 (SD = 0.824) | 0.4526 |

ANOVA shows that there was no significant difference in AHS or ABG protein expression amongst the three groups, i.e. the normal, the active and the chronic toxoplasmosis.

1) Fold change measures the degree of change in the protein of the T. gondii-infected individuals, compared to that of normal controls (n = 10). This is measured by dividing the average spot intensity in the infected persons by the average spot intensity in the controls. The t-test showed that there are significant differences in AHS and ABG protein expression between serum from patients (active or chronic toxoplasmosis) and normal serum.

sera. This enabled identification of three antigenic proteins from T. gondii namely a hypothetical protein chrXII: 3984434-3 TGME 49 (29 kDa); dual specificity protein phosphatase, catalytic domain (33 kDa) and NADPH-cytochrome p450 reductase (38 kDa). These three proteins may thus be potentially useful as candidates for the development of an antigen detection test for toxoplasmosis. To the best of our knowledge, this is the first report of MS/MS identification of circulated proteins in the sera samples of active toxoplasmosis patients. We attempted to use purified polyclonal rabbit anti-T. gondii IgG as the primary antibody in the immunoblotting, but the background of the developed films was too intense and thus not useful (blots not shown). This may be caused by non-specific binding, because a lot of other interfering proteins and antibodies may exist in the rabbit serum sample. The same observations were made when we tried using purified rabbit anti-T. gondii IgG as the capture antibody in two different CA-ELISA formats [12].

We have also identified host-specific proteins (AHS and ABG) from the sera of active toxoplasmosis patients who were immunogenic in Western blots. AHS and ABG may have an important role in host–parasite interactions, especially in the mechanism of adhesion and invasion of T. gondii into host cells. Previous studies have shown AHS or fetuin A as a highly sialylated glycoprotein [30] and was reported to be involved in host cell invasion by T. gondii [31]. The interaction between T. gondii and the embryonic glycoprotein fetuin A or AHS was first reported by Gross et al. [32]. In their study, by using AHS-agarose chromatography of T. gondii tachyzoites extracts, they had identified an AHS-binding protein of 15 kDa that specifically recognised AHS glycan structures. Our finding, which showed the immunoreactivity of AHS from the sera of active toxoplasmosis patients, is thus in agreement with their results. Thus, we hypothesised that binding of AHS to the 15 kDa tachyzoites antigen may have resulted in the immunoreactivity of AHS in the sera of actively infected individuals. This is indirectly corroborated by the difference in score and additional unmatched peptides/spectrum of AHS when MS data comparisons were made between sera of normal and active infection. Although AHS and ABG were found to have increased expression in the sera of both active and chronic patients, they only showed antigenicity in Western blots of sera from active infection. This result suggested the potential of host-specific proteins to be the indicator for this disease progression. Currently, further work is being performed to confirm the interaction of the T. gondii proteins/peptides with AHS and ABG.

Binding of AHS to diverse ligands in vitro has been reported [33]. In host–parasite interaction, AHS has been shown to play an important role in sporozoites infectivity of Plasmodium berghei in hepatocyte, by binding to thrombospondin-related adhesive protein (TRAP). TRAP belongs to type-1 transmembrane protein family with a long extracellular region, consisting of two adhesive domains: A domain and a thrombospondin repeat [34]. In Toxoplasma spp., an adhesive protein, MIC2 (a secretory protein that belongs to TRAP family member) is expressed in all invasive stages only, thus indirectly suggesting that this protein may be essential for parasite invasion [35]. Moreover, MIC2 has also been shown to be conserved across the Apicomplexa
family [35]; therefore, further supporting the possible functional roles of MIC2 and also other TRAP family proteins in the parasites invasion into host cells. Interestingly, in preliminary experiments using similar strategy and serum from patients with other parasitic infections (filariasis and amoebiasis), AHS also showed increased expression and immunogenicity when probed with the corresponding primary antibodies. However, this was not the case with serum samples from leishmaniasis and amoebiasis patients (results not shown). These observations suggested that AHS may be an important indicator of parasitic infection. With regard to ABG, our study is the first to report its possible role in patients with toxoplasmosis. It has never been reported in relation to any parasitic disease or other infectious diseases. Therefore, the role of ABG protein in patients with active toxoplasmosis remains to be investigated.

In conclusion, 2-DE followed by Western blotting has enabled the identification of three circulating T. gondii proteins and highlights the potential of these proteins and host-specific proteins. AHS and ABG, to serve as infection markers for both active and/or chronic toxoplasmosis.

We thank Associate Professor Dr. Gam Lay Harn for technical advice rendered in the initial phase of the study. This research is supported by research grants from MOSTI Science Fund [No. 02-01-05-SF0154 (USM No. 305/CIPPM/613206)] and USM Research University (RU) fund (No. 1001/CIPPM/8130132).

The authors have declared no conflict of interest.

5 References

[1] Tenter, A. M., Heckerath, A. R., Weiss, L. M., Int. J. Parasitol. 2000, 30, 1217–1258.
[2] Dubey, J. P., Beattie, C. P., Toxoplasmosis of Animals and Man, CRC Press: Boca Raton, FL 1998.
[3] Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., McAuley, J. B., Am. J. Epidemiol. 2001, 154, 357–365.
[4] Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., McAuley, J. B., Am. J. Epidemiol. 2001, 154, 357–365.
[5] Susanto, L, Muljono, R., Southeast Asian J. Trop. Med. Public Health 2001, 32, 195–197.
[6] Attallah, A. M., Ismail, H., Ibrahim, A. S., J. Immunoassay Immunoch.m. 2006, 27, 45–60.
[7] He, Q. Y., Lau, G. K. K., Zhou, Y., Yuen, S. T., Lin, M. C., Kung, H. F., Chiu, J. F., Proteomics 2003, 3, 666–674.
[8] Lee, N. I., Chen, C. H., Sheu, J. C., Lee, H. S., Huang, G. T., Chen, D. S., Yu, C. Y., Wen, F. J., Chow, L. P., Proteomics 2006, 6, 2865–2873.
[9] Gangadharan, B., Anurobas, R., Dwik, R. A., Zitzmann, N., Clin. Chem. 2007, 53, 1792–1799.
[10] He, Q. Y., Yang, H., Benjamin, C. Y. W., Chiu, J. F., Dig. Dis. Sci. 2008, 53, 3112–3118.
[11] Thayan, R., Huat, T. L., See, L. L. C., Tan, C. P. L., Kairurullah, N. S., Yusof, R., Devi, S., Trans. R. Soc. Trop. Med. Hyg. 2009, 103, 413–419.
[12] Emelia, O., Zeehaidia, M., Sulaiman, O., Rohela, M., Saadatnia, G., Chen, Y., Rahmah, N., J. Immunoassay Immunoch.m. 2010, 31, 79–91.
[13] Chen, Y., Lim, B. K., Peh, S. C., Abdul-Rahman, P. S., Hashim, O. H., Proteome Sci. 2008, 6, 20.
[14] Heukeshoven, J., Dernick, R., Electrophoresis 1988, 9, 28–32.
[15] Shevchenko, A., Wilm, M., Vorm, O., Mann, M., Anal. Chem. 1996, 68, 850–858.
[16] Nischik, N., Schade, B., Dlugonska, H., Reichmann, G., Fischer, G., Microbes Infect. 2001, 3, 689–699.
[17] Gastens, M. H., Fischer, H. G., Int. J. Parasitol. 2002, 32, 1225–1234.
[18] Neudwck, A., Stachelhaus, S., Nischik, N., Striepen, B., Microbes Infect. 2002, 4, 581–590.
[19] Reichmann, G., Dlugonska, H., Hiszczyznska-Sawicka, E., Fischer, H., Microbes Infect. 2001, 3, 779–787.
[20] Nielsen, H. V., Schmidt, D. R., Petersen, E., J. Clin. Microbiol. 2005, 43, 711–715.
[21] Ma, G. Y., Zhang, J. Z., Yin, G. R., Zhang, J. H., Exp. Parasitol. 2009, 122, 41–46.
[22] Nielsen, P., Krogh, A., Bioinformatics 2005, 21, 4322–4329.
[23] Guigo, R., Flicek, P., Abril, J. F., Reymond, A., Lagarde, J., Denoeud, F., Antonarakis, S., Ashburner, M., Bajic, V. B., Birney, E., Castelo, R., Eyras, E., Ucla, C., Gingeras, T. R., Harrow, J., Hubbard, T., Lewis, S. E., Reese, M. G., Genome Biol. 2006, 7, S21–S31.
[24] Lu, F., Jiang, H., Ding, J., Mu, J., Valenzuela, J. G., BMC Genomics 2007, 8, 255.
[25] Dybas, J. M., Madrid-Aliste, C. J., Che, F.-Y., Nieves, E., Rykunov, D., Angeletii, R. H., Weiss, L. M., Kim, K., Fischer, A., PLoS ONE, 2008, 3, e3899.
[26] Gladkova, S. V., Dedkova, L. M., Belanov, E. F., Gibliatnikova, A. V., Kiseleva, Z. F., Reshetnikov, S. S., Parasitology 2000, 4, 15–18.
[27] Araujo, F. G., Handeman, E., Remington, J. S., Infect. Immun. 1980, 30, 12–16.
[28] Maria, G., Cecilia, G. V., Rafael, S. D., Alfonso, I. R., Rev. Bras. Med. Trop. 1998, 31, 271–277.
[29] Shojaee, S., Keshavarz, H., Rezaian, M., Mohebali, M., Pak. J. Med. Sci. 2007, 23, 100–102.
[30] Vonlaufen, N., Naguleswaran, A., Gianinazzi, C., J. Med. Microbiol. 2001, 50, 153–159.
[31] Monteiro, V. G., Soares, C. P., de Souza, W., EMBO Microbiol. Lett. 1999, 164, 323–327.
[32] Gross, U., Hambach, C., Windeck, T., Heesemann, J., Parasitol. Res. 1993, 79, 191–194.
[33] Jethwaney, D., Lepore, T., Hassan, S., Mello, K., Infect. Immun. 2005, 73, 5883–5891.
[34] Girma, J. P., Meyer, D., Verweij, C. L., Pannekoek, H., Sixma, J. J., Blood 1987, 70, 605–611.
[35] Huynh, M. H., Rabenau, K. E., Harper, J. M., Beatty, W. L., Sibley, L. D., Carruthers, V. B., EMBO J. 2003, 22, 2082–2090.