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Short communication

Proteomic patterns as biomarkers for the early detection of schistosomiasis japonica in a rabbit model

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ABSTRACT

Objective: The objective of this study was to identify proteomic patterns in sera for the early detection of *Schistosoma japonicum* infections in a rabbit model. Proteomic patterns were to be established by profiling serum proteins using magnetic bead (MB) separation and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).
Methods: Forty rabbits were randomly allocated to two groups. One group was infected with 1500 *S. japonicum* cercariae, the other served as non-infected control. An additional group of *Toxoplasma gondii*-infected rabbits served as specificity control group. Sera were obtained from each rabbit once a week post-infection and were subject to weak cation exchange beads (MB-WCX) treatment, followed by MALDI-TOF MS analysis. The proteomic pattern of infected and control rabbits was established 7 weeks post-infection with the ClinProTool MS data analysis program.
Results: Seven peaks with a clear difference in amplitude between the infected and control groups were detected, 4 peaks with mass charge ratio (m/z) of 1787, 2834, 3484 and 3531 were up-regulated and 3 peaks with a m/z of 1715, 3151 and 4018 were down-regulated in infected rabbits. The established diagnostic proteomic pattern was highly sensitive and specific. In weeks 1–4 post-infection, characteristic proteomic patterns could be detected in 30%, 55%, 75% and 80% of the infected rabbits, whereas ELISA testing resulted in positive results from week 3 onwards. All *T. gondii* control sera were classified *S. japonicum* negative.
Conclusions: MALDI-TOF MS coupled with MB separation enables early, rapid and accurate diagnosis of schistosomiasis in a rabbit model.

1. Introduction

Schistosomiasis is one of the most common and serious parasitic diseases in humans, affecting more than 200 million people with 779 million individuals at risk of infection worldwide, and causing the loss of an estimated 4.5 million disability-adjusted life years [1,2]. Along with recent progress in the treatment, prevention and control of schistosomiasis [3], both prevalence and infection intensity have decreased significantly in areas with an established control system, such as the People's Republic of China (P.R. China) where the species *Schistosoma japonicum* is endemic [4,5]. Reliable diagnosis, especially of the early stages of infection, is crucial for the control of schistosomiasis. Currently used conventional diagnostic procedures have either low sensitivity for the detection of early and low-intensity infections (parasitological methods such as Kato–Katz) [6,7], low specificity (serological tests) [7] or are time-consuming (parasitological methods such as the hatching test) [8]. Immunodiagnostic assays are mainly based on the detection of soluble egg antigen (SEA) and may fail to distinguish schistosomiasis from other parasite infections such as *Paragonimus proliferus* [9], *Clonorchis sinensis* [10–12] and *Toxoplasma gondii* [13,14]. Additionally, immunological assays cannot be used for early diagnosis of acute schistosomiasis since antibodies only emerge after two weeks of infection [15], and host anti-*S. japonicum* antibodies usually can be detected for 1 year or more after an infection has been cleared, rendering the reliable diagnosis of active infections difficult. False positive results may lead to unnecessary or inappropriate chemotherapy.

We aimed at developing a sensitive mass spectrometry (MS)-based strategy for the early diagnosis of schistosomiasis [16,17]. Detecting protein markers by MS profiling has numerous advantages including high sensitivity, short duration from sample

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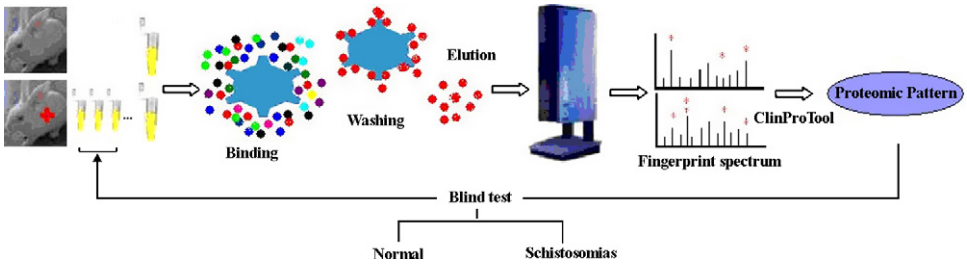

Fig. 1. Principle of sample profiling using MALDI-TOF MS. The rabbits were stratified into two groups, a control group (*upper* picture) and an infected group (*lower* picture). 1500 *Schistosoma japonicum* cercariae were used for infection. MB-WCX beads combined with MALDI-TOF MS were used after serum collection, the resulting spectra were analyzed with the ClinProTool software to establish the proteomic pattern.

collection to diagnosis, potential for high-throughput screening and requirement for only small biological samples [18]. Such approaches have already been successfully applied for the detection of diseases including parasitic diseases such as Chagas diseases (*Trypanosoma cruzi*) [19] and *Entamoeba histolytica* [20], as well as in rheumatoid arthritis screening [21] and cancer research [22–25]. Following these examples, we adapted the approach and developed MB-WCX separation (magnetic beads based weak cation exchange chromatography was developed for enrichment and purification of peptides and proteins from complex biological samples) and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) assay for the early and sensitive detection of schistosomiasis japonica infections.

2. Materials and methods

2.1. Parasites and laboratory animals

S. japonicum (Chinese strain) cercariae were obtained from infected *Oncomelania hupensis* snails (its intermediate host) bread at the Jiangsu Institute of Parasitic Diseases (JIPD) in Wuxi, P.R. China using standard procedures [26,27]. Fouty adult, healthy, male and female New Zealand rabbits with body weights of 2.0–3.0 kg were employed in the experiments which were carried out at the experimental animal facility of JIPD. The rabbits were randomly assigned to two groups, a control group and an infected group. The infection with 1500 *S. japonicum* cercariae was through the shaved abdominal skin and no other treatment or manipulation of the animals was involved. The control group was exposed to physiological saline at similarly shaved abdominal skin [26,27].

2.2. Serum collection, ELISA and fecal examination

Once every week, a blood sample was collected from the ear vein of all infected and non-infected rabbits. The sample was left to clot for 1 h at room temperature, and centrifuged at 3000 × *g* for 10 min at 4 °C to obtain serum. All sera aliquots were stored at −80 °C pending use.

Successful *S. japonicum* infection was ascertained using an enzyme-linked immunosorbent assay (ELISA) according to a previously published protocol [28,29]. In brief, adult worm antigen (AWA, produced at Jiangsu Institute of Parasitic Diseases) was diluted with carbonate buffer (pH 9.6) to 5 μg/ml. Aliquots of 100 μl were pipetted in wells and the plates stored overnight at 4 °C. Plates were then washed thrice with phosphate buffered solution (PBS) containing 0.05% Tween-20 (PBST) and blocked with 0.3% (w/v) bovine serum albumin (BSA, Sigma) in PBS for 1 h at room temperature. After blocking, plates were again washed thrice with PBST before incubation for 1 h at 37 °C with rabbit sera diluted 1:20 with PBS for IgG detection. Rabbit sera obtained before infection were used as negative controls. The plates were then washed five times with PBST, followed by incubation with 1:5000 horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Sigma, USA) for 1 h at 37 °C. After this step, plates were again washed five times with PBST before addition of tetramethyl benzidine (TMB) substrate (Jingmei Biotech, P.R. China) for 5–10 min. The reaction was stopped by the addition of sulphuric acid (2 mol/l) and the optical density (OD) read at 450 nm using a Microplate Reader (Autobio, Zhengzhou, China). The cut-off value was calculated as the OD of the sample divided by the OD of the negative control ≥2.1.

Stool samples were also collected every week after infection, and examined by the Kato–Katz thick smear method as previously described [8].

2.3. MALDI-TOF

The set-up of the study is depicted in Fig. 1. Magnetic beads-based weak cation exchange chromatography (MB-WCX, Bruker Daltonics, Bremen, Germany) was used according to the standard protocol. Serum samples were thawed on ice and centrifuged at 4 °C (12,000 × *g* for 10 min). 10 μl of the supernatant was added to 10 μl binding buffer (Bruker Daltonics, Bremen, Germany) and 10 μl MB-WCX beads in a standard thin-walled PCR-tube. After mixing and 5 min incubation at room temperature, the tubes were placed on the magnetic separator (Bruker Daltonics, Bremen, Germany). The beads were carefully washed thrice with 100 μl washing buffer. The fraction collected after incubation with 5 μl elution buffer for 2 min was transferred into a fresh tube containing 5 μl stabilization buffer, and stored at −20 °C.

A linear MALDI-TOF MS (Auto flex III, Bruker Daltonics, Bremen, Germany) was used for spectrum acquisition with default settings and a linear positive mode, named LP_Clinprot.par.

Mass accuracy was calibrated before measurements using the standard calibration mixture peptide I and protein I, mixed 1:1 by volume (Bruker Daltonics, Bremen, Germany). For parameter setting, mass spectra were acquired in the 1000–12,000 *m*/*z* range. Targets were prepared by spotting 1 μl of a mixture containing 10 μl of 0.3 g l^{-1} HCCA (α-cyano-4-hydroxycinnamic acid) in ethanol–acetone (2:1 by volume) and 1 μl of the eluted proteome fraction on the MTP-AnchorChip 800/384 target (Bruker Daltonics, USA). Four MALDI spots of each sample were measured. For each spot, 500 spectra were acquired (100 laser shots at 5 different spot positions). All spectra with a signal-to-noise (S/N) ratio > 3 were recorded with the FlexControl software (Bruker Daltonics, Bremen, Germany).

2.4. Data processing and analysis

ClinProTools is designed to facilitate the processing and comparison of multiple spectra by automatically peak defining, normalizing, baseline subtracting, and recalibrating the data. All spectra (control and infection group) were imported into ClinProTool software for spectrum post-processing. The result of this data preparation was a collection of normalized peak amplitude

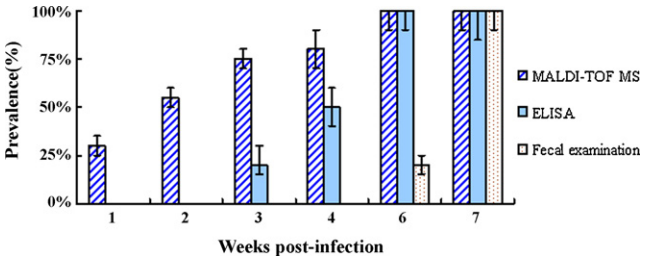

Fig. 2. Measured *S. japonicum* prevalence among the infected rabbits according to diagnostic method, measured in weekly intervals.

for each spectrum. Then these processed spectra were used, in a supervised training, to build a model describing a proteomic fingerprint that can discriminate infected samples from normal. Model building was performed by selecting a small subset of relevant peaks and establishing clusters using the amplitude of these peaks. Most of the parameters of this Bruker algorithm were automatically determined to optimize the performance of the peak selection. The result of the peak selection algorithm is the peak combination (proteomic fingerprint) that proved to separate best between the different classes. The aim of model building in supervised training was to describe the training data in such a way that new data (test data) could be classified afterwards [30]. The ClinProTool software (Ver. 2.0, Bruker Daltonics) was employed to identify corresponding peaks in two groups of spectra, i.e. infected and control animals, and to establish the proteomic pattern for the detection of schistosomiasis with genetic algorithms. Statistical analysis relied on Welch's *t*-test. A P value < 0.05 was considered significant and one < 0.01 highly significant.

Once the proteomic pattern had been established, the output value for healthy control animals was set as 0, that for infected animals as 1. Samples collected 1–4 weeks post-infection were scored "0" or "1" without prior knowledge of their true infection status.

2.5. Reproducibility and variability of proteomic pattern, and species specificity

The reproducibility and efficiency of MB-WCX fractionation were assessed by measuring all samples thrice. To evaluate the analytical variability, ten bead preparations of the peptide/protein calibration mixture and the sera samples were analyzed within one day (day variability) and within three consecutive days (day to day variability).

The specificity of the differential peaks for schistosomiasis was assessed by establishing whether the same proteomic patterns could be detected in sera from 15 *Toxoplasma gondii*-positive rabbits (from JIPD).

3. Results

3.1. S. japonicum infection rate and clinical characteristics of the cercariae-exposed rabbits

ELISA and fecal examination were used to confirm infections among the rabbits exposed to *S. japonicum* cercariae. As shown in Fig. 2, every rabbit from the group exposed to cercariae was serologically and coprologically confirmed positive seven weeks post-infection.

Infected rabbits could be classified as two stages. Stage I (0–2 weeks post-infection) with no significant change compared to non-infected animals, and stage II, when infected rabbits become weak and representative clinical signs could be seen.

Table 1
Selected peak amplitudes in sera from control and *S. japonicum*-infected rabbits obtained using MALDI-TOF MS combined with MB-WCX profiling. Analysis: ClinProTool.

Peak (m/z)	Peak amplitude		P value
	Control	Infected	
1715	335.84 ± 88.92	125.24 ± 50.25	<0.05
1787	1355.26 ± 188.27	3789.16 ± 770.54	<0.01
2834	1256 ± 405.22	4012 ± 788.45	<0.01
3151	679 ± 123.02	237.17 ± 100.25	<0.05
3484	212.77 ± 88.16	565.23 ± 138.56	<0.01
3531	278.56 ± 56.01	482.15 ± 99.98	<0.05
4018	366.78 ± 95.77	105.99 ± 67.56	<0.01

Note: Data are expressed as mean ± SD.

3.2. Proteomic pattern established by MALDI-TOF

A sample has been obtained right after infection (0 days) and has been measured. No difference between controls and infected animals was found. The intensities of several peaks were significantly different upon comparison of the infected and control group (Table 1). In the mass range 1000–12,000 Da, a proteomic pattern composed of seven statistically different protein peaks was established which allowed distinguishing the control group from the infected group seven weeks post-infection, as shown in Fig. 3A and B. Among the seven peaks, three peaks with m/z 1715, 3151 and 4018, respectively were down-regulated in infected animals, while the other four peaks with m/z 1787, 2834, 3484 and 3531 were up-regulated. The peak amplitudes were 2–3 fold, as shown in Fig. 3C–F. The results of the statistical analysis showing P values of several peaks are shown in Table 1 and Box–Whisker plots are provided in the supplementary material (Sup. 1). The reproducibility of the results was good (data not shown).

3.3. Comparison of diagnostic approaches for early detection of a schistosomiasis japonica infection, and species specificity

The peak patterns established with MS seven weeks post-infection were used to analyze serum samples collected at earlier infection stages. As shown in Fig. 2, MS analysis correctly identified 30% (1 week post-infection), 55% (2 weeks), 75% (3 weeks) and 80% (4 weeks) of the samples as positive whereas the conventional ELISA detected 0, 0, 20% and 50% of the infections, respectively. Eggs were first found in fecal samples six weeks post-infection.

All sera from *T. gondii*-infected rabbits were classified "0" (=not infected) using the criteria for *S. japonicum* infection detection established by MS (*Sup. 2*).

4. Discussion

For *S. japonicum* diagnosis, both immunological methods such as ELISA which detect host antibodies and microscopic methods for the direct visualization of parasite eggs in feces are currently used. However, there are limitations to these two methods with regard to sensitivity and specificity. Molecular biology techniques such as PCR-ELISA have also been investigated for the direct diagnosis of schistosomiasis [31], and proved suitable to detect schistosome DNA. We have established a new method to detect schistosomiasis infections using MALDI-TOF MS, which not only provides a robust proteomic pattern for the early detection of infections, but also facilitates the study of biomarkers for *S. japonicum* infections (Table 2).

Proteomics-based approaches using highly sensitive MALDI-TOF MS for the early and reliable diagnosis of infections and diseases are promising and have been used to identify biomarkers in biological samples such as serum [32,33], urine [34], cere-

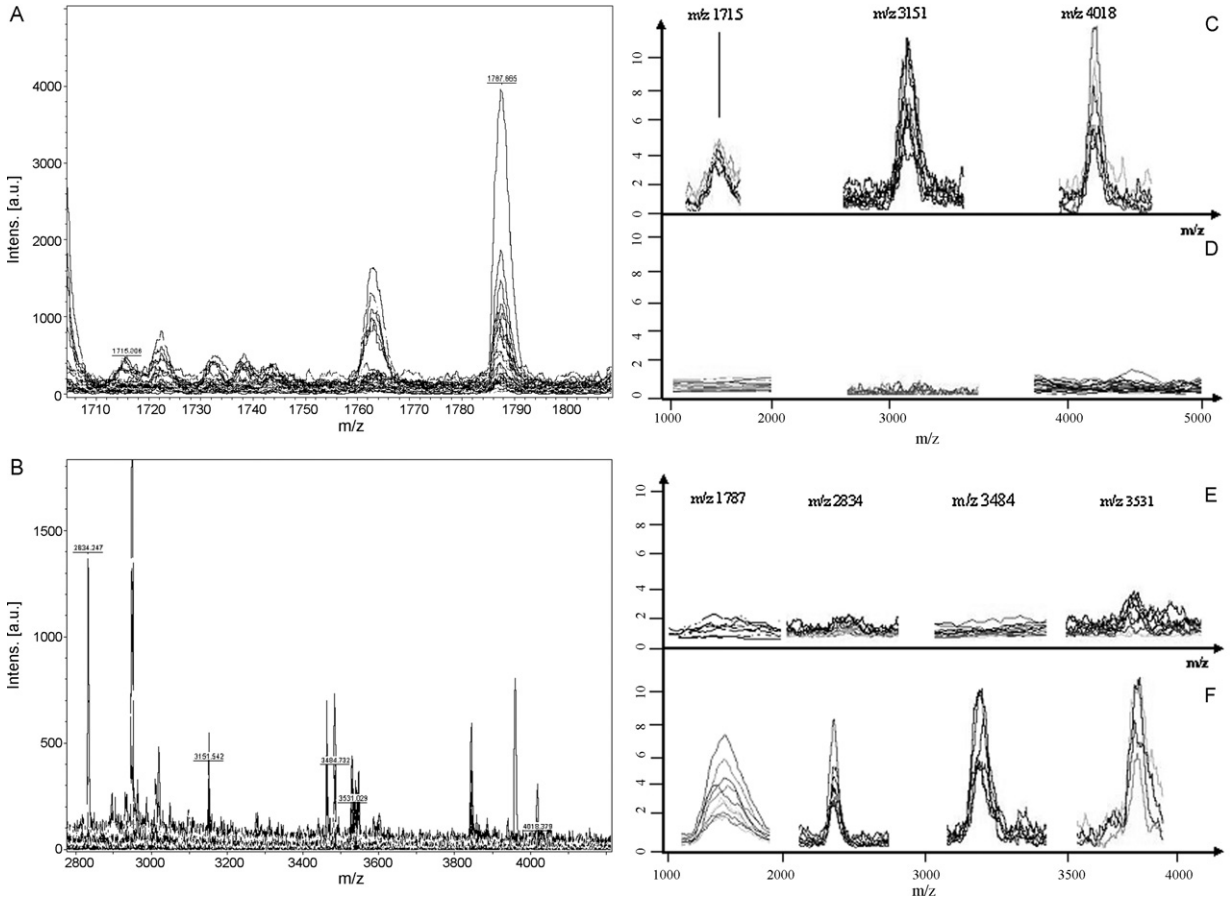

Fig. 3. Mass spectra of 20 control and 20 *S. japonicum*-infected rabbit sera obtained using MB-WCX bead fractionation and MALDI-TOF-MS. (A and B) MALDI-TOF MS spectra of sera from control and infected rabbits overlaid. (C and E) Non-infected rabbits. (D and F) *S. japonicum*-infected rabbits. The MS profiles show down-regulation of *m*/*z* 1715, 3151, 4018 (C and D) and up-regulation of *m*/*z* 1787, 2834, 3484, 3531 (E and F) in sera from infected rabbits seven weeks post-infection.

brospinal fluid [35,36], saliva [37,38] and tears [39]. With regard to schistosomiasis, MS has already been used for biomarker detection. Ecdysteroids produced by adult schistosomes can be detected in sera of infected hosts and thus can be used for diagnosis [40]. Gas chromatography/MS has been used to investigate the monosaccharide content of the ethanol-soluble moiety of crude polysaccharide fractions of the tapeworm *Hymenolepis diminuta* and *S. mansoni* [41]. However, the employed methods are technically challenging and time-consuming, and hence not suitable for routine diagnosis. Small molecule markers have also been identified using MALDI-TOF MS in 11 urine samples collected from Egyptians with *S. mansoni* infections of different intensity using MALDI-TOF MS [34]. The method employed to detect schistosomiasis in our study differs from the previous study; spectra from samples pertaining to two groups (infected and non-infected) were obtained, and the proteomic pattern was found to discriminate between the two groups. Thus, the method described here represents a promising new approach to further explore the molecular characteristics and changes associated with schistosomiasis.

Table 2
Sensitivity and specificity of the proteomic pattern in rabbit sera established by MALDI-TOF MS.

Group	*n*	Predicted infected	Predicted not infected	Accuracy
Infected[a]	20	19	1	95.0% (sensibility)
Control	20	0	20	100.0% (specificity)

[a] Seven weeks post-infection.

In this study we examined differential proteins expression patterns in rabbit serum with MB-WCX beads and MALDI-TOF. Seven peaks with a clear difference in amplitude between the two groups were detected, and a proteomic pattern to discriminate the infected and non-infected was founded. The sensitivity of the MALDI-TOF MS for the detection of *S. japonicum* infections in rabbits was 95%. As expected, the MALDI-TOF MS was more sensitive than conventional ELISA and fecal examination for schistosomiasis diagnosis, and allowed the detection of infections as early as one week post-infection. The first positive ELISA test result was obtained 3 weeks post-infection and the first eggs were found 6 weeks after the exposure of the rabbits to cercariae. The specificity was 100% both when sera from *S. japonicum*-infected and non-infected rabbits were compared and when sera from *T. gondii*-infected rabbits were assessed. The ClinProTools software proved to be a suitable tool for the analysis of the data derived from MALDI-TOF MS.

It must be noted that the established biomarker patterns for schistosomiasis japonica diagnosis cannot be directly translated to other species, e.g., humans or livestock, without calibration. It is high probable that each species will show unique schistosomiasis-specific diagnostic MS patterns. Furthermore, the amplitude of the seven peaks is too low to be detected by MS/MS which could reveal sequences of these peptides. We are currently working on separating the differential peaks we observed for MS/MS. Once this has been achieved, we believe that the origin and true nature of the peaks could be studied. We conclude that this study has established the principle for MALDI-TOF MS diagnosis of *S. japonicum* and we expect that with further adaptation, this approach could also be deployed for the diagnosis of schistosome infections in humans.

Conflict of interest statement

All authors declare they have no conflict of interest.

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Ethics statement

All experiments described here fully complied with the current laws of the country in which the experiments were performed (P.R. China).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.10.013.

References

[1] P. Steinmann, J. Keiser, R. Bos, M. Tanner, J. Utzinger, Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk, Lancet Infect. Dis. 6 (2006) 411–425.
[2] P.J Hotez, D.H. Molyneux, A. Fenwick, E. Ottesen, S. Ehrlich Sachs, J.D. Sachs, Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria, PLoS Med. 3 (2006) e102.
[3] B. Gryseels, K. Polman, J. Clerinx, L. Kestens, Human schistosomiasis, Lancet 368 (2006) 1106–1118.
[4] X.N. Zhou, L.Y. Wang, M.G. Chen, T.P. Wang, J.G. Guo, X.H. Wu, Q.W. Jiang, J. Zheng, X.Y. Chen, An economic evaluation of the national schistosomiasis control programme in China from 1992 to 2000, Acta Trop. 96 (2005) 255–265.
[5] J. Utzinger, X.N. Zhou, M.G. Chen, R. Bergquist, Conquering schistosomiasis in China: the long march, Acta Trop. 96 (2005) 69–96.
[6] D. Engels, E. Sinzinkayo, B. Gryseels, Day-to-day egg count fluctuation in *Schistosoma mansoni* infection and its operational implications, Am. J. Trop. Med. Hyg. 54 (1996) 319–324.
[7] M.J. Doenhoff, A.E. Butterworth, R.J. Hayes, R.F. Sturrock, J.H. Ouma, D. Koech, M. Prentice, J. Bain, Seroepidemiology and serodiagnosis of schistosomiasis in Kenya using crude and purified egg antigens of schistosoma-mansoni in Elisa, Trans. R. Soc. Trop. Med. Hyg. 87 (1993) 42–48.
[8] J.M. Yu, S.J. de Vlas, Q.W. Jiang, B. Gryseels, Comparison of the Kato–Katz technique, hatching test and indirect hemagglutination assay (IHA) for the diagnosis of Schistosoma japonicum infection in China, Parasitol. Int. 56 (2007) 45–49.
[9] W.L Wang, J.S. Tang, D.G. Xia, X.Z. Zhang, A preliminary study on specific adult-antigens of paragonimus proliferus, Chin. J. Parasit. Dis. Control 2 (2001) 19–20.
[10] A. Ishii, H. Matsuoka, T. Aji, H. Hayatsu, Y. Wataya, S. Arimoto, H. Tokuda, Evaluation of the mutagenicity and the tumor-promoting activity of parasite extracts: Schistosoma japonicum and Clonorchis sinensis, Mutat. Res. 224 (1989) 229–233.
[11] S. Kojima, M. Yokogawa, T. Tada, Production and properties of reaginic antibodies in rabbits infected with Clonorchis sinensis or Schistosoma japonicum, Exp. Parasitol. 35 (1974) 141–149.
[12] M. Itoh, S. Sato, Multi-dot enzyme-linked immunosorbent assay for serodiagnosis of trematodiasis, Southeast Asian J. Trop. Med. Public Health 21 (1990) 471–474.
[13] S. He, E. Yu, Identification of serology cross reaction between acute schistosomiasis and toxoplasma, Chin. J. Zoonoses 9 (1993) 5–6.
[14] R. Chen, S.H. Lu, D. Lou, A.F. Lin, X.J. Zeng, Z.Y. Ding, L.Y. Wen, N.B. Ohta, J.F. Wang, C. Fu, Evaluation of a rapid ELISA technique for detection of circulating antigens of Toxoplasma gondii, Microbiol. Immunol. 52 (2008) 180–187.
[15] N. Sandoval, M. Siles-Lucas, J. Lopez Aban, J.L. Perez-Arellano, T. Garate, A. Muro, Schistosoma mansoni: a diagnostic approach to detect acute schistosomiasis infection in a murine model by PCR, Exp. Parasitol. 114 (2006) 84–88.
[16] L.D. Wang, H.G. Chen, J.G. Guo, X.J. Zeng, X.L. Hong, J.J. Xiong, X.H. Wu, X.H. Wang, L.Y. Wang, G. Xia, Y. Hao, D.P. Chin, X.N. Zhou, A Strategy to control transmission of Schistosoma japonicum in China, N. Engl. J. Med. 360 (2009) 121–128.
[17] X.H. Zeng, H.Q. Huang, D.S. Chen, H.W. Jin, H.Y. Huang, Proteomic study of serum using gel chromatography and MALDI-TOF MS reveals diagnostic biomarkers in male patients with liver-cancer, Int. J. Mass Spectrom. 261 (2007) 108–114.
[18] R. Ketterlinus, S.Y. Hsieh, S.H. Teng, H. Lee, W. Pusch, Fishing for biomarkers: analyzing mass spectrometry data with the new ClinProTools software, Biotechniques (Suppl.) (2005) 37–40.
[19] M. Ndao, T.W. Spithill, R. Caffrey, H. Li, V.N. Podust, R. Perichon, C. Santamaria, A. Ache, M. Duncan, M.R. Powell, B.J. Ward, Identification of novel diagnostic serum biomarkers for Chagas' disease in asymptomatic subjects by mass spectrometric profiling, J. Clin. Microbiol. 48 (2010) 1139–1149.
[20] A. Makioka, M. Kumagai, S. Kobayashi, T. Takeuchi, Differences in protein profiles of the isolates of Entamoeba histolytica and E. dispar by surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF MS) ProteinChip assays, Parasitol. Res. 102 (2007) 103–110.
[21] L. Long, R. Li, Y. Li, C. Hu, Z. Li, Pattern-based diagnosis and screening of differentially expressed serum proteins for rheumatoid arthritis by proteomic fingerprinting, Rheumatol. Int. (2010), doi:10.1007/s00296-010-1407-4.
[22] J. Villanueva, D.R. Shaffer, J. Philip, C.A. Chaparro, H. Erdjument-Bromage, A.B. Olshen, M. Fleisher, H. Lilja, E. Brogi, J. Boyd, M. Sanchez-Carbayo, E.C. Holland, C. Cordon-Cardo, H.I. Scher, P. Tempst, Differential exoprotease activities confer tumor-specific serum peptidome patterns, J. Clin. Invest. 116 (2006) 271–284.
[23] Y. Guo, K. Shibuya, G. Cheng, K.Q. Rao, L.M. Lee, S.L. Tang, Tracking China's health reform, Lancet 375 (2010) 1056–1058.
[24] M.A. Karpova, S.A. Moshkovskii, I.Y. Toropygin, A.I. Archakov, Cancer-specific MALDI-TOF profiles of blood serum and plasma: biological meaning and perspectives, J. Proteomics 73 (2010) 537–551.
[25] W. Pusch, M. Kostrzewa, Application of MALDI-TOF mass spectrometry in screening and diagnostic research, Curr. Pharm. Des. 11 (2005) 2577–2591.
[26] S.R. Smithers, R.J. Terry, The infection of laboratory hosts with cercariae of Schistosoma mansoni and the recovery of the adult worms, Parasitology 55 (1965) 695–700.
[27] F. Lewis, Schistosomiasis, Curr. Protoc. Immunol. (2001) 11, Chapter 19, Unit 19.
[28] T. Suzuki, Y. Osada, T. Kumagai, A. Hamada, E. Okuzawa, T. Kanazawa, Early detection of Schistosoma mansoni infection by touchdown PCR in a mouse model, Parasitol. Int. 55 (2006) 213–218.
[29] Y. Dai, Y. Zhu, D.A. Harn, X. Wang, J. Tang, S. Zhao, F. Lu, X. Guan, DNA vaccination by electroporation and boosting with recombinant proteins enhances the efficacy of DNA vaccines for Schistosomiasis japonica, Clin. Vaccine Immunol. 16 (2009) 1796–1803.
[30] X. Zhang, S.M. Leung, C.R. Morris, M.K. Shigenaga, Evaluation of a novel, integrated approach using functionalized magnetic beads, bench-top MALDI-TOF-MS with prestructured sample supports, and pattern recognition software for profiling potential biomarkers in human plasma, J. Biomol. Tech. 15 (2004) 167–175.
[31] L.I. Gomes, L.H. Dos Santos Marques, M.J. Enk, M.C. de Oliveira, P.M. Coelho, A. Rabello, Development and evaluation of a sensitive PCR-ELISA system for detection of schistosoma infection in feces, PLoS Negl. Trop. Dis. 4 (2010) e664.
[32] M.E. de Noo, R.A.E.M. Tollenaar, A. Ozalp, P.J.K. Kuppen, M.R. Bladergroen, P.H.C. Eilers, A.M. Deelder, Reliability of human serum protein profiles generated with C8 magnetic beads assisted 18 MALDI-TOF mass spectrometry, Anal. Chem. 77 (2005) 7232–7241.
[33] S.M. Roy, M. Anderle, H. Lin, C.H. Becker, Differential expression profiling of serum proteins and metabolites for biomarker discovery, Int. J. Mass Spectrom. 238 (2004) 163–171.
[34] M.L.M. Robijn, J. Planken, D. Kornelis, C.H. Hokke, A.M. Deelder, Mass spectrometric detection of urinary oligosaccharides as markers of Schistosoma mansoni infection, Trans. R. Soc. Trop. Med. Hyg. 102 (2008) 79–83.
[35] J.Y. Pan, Q. Wang, Q. Shao, Y.Y. Cheng, H.B. Qu, Development and validation of a column-switching LC-ESI-MS assay for determination of huperzine A in rat plasma and cerebrospinal fluid, Chromatographia 69 (2009) 445–451.
[36] T. Waybright, A.M. Avellino, R.G. Ellenbogen, B.J. Hollinger, T.D. Veenstra, R.S. Morrison, Characterization of the human ventricular cerebrospinal fluid proteome obtained from hydrocephalic patients, J. Proteomics 73 (2010) 1156–1162.
[37] F. Chevalier, C. Hirtz, S. Chay, F. Cuisinier, N. Sommerer, M. Rossignol, D. de Périère, Proteomic studies of saliva: a proposal for a standardized handling of clinical samples, Clin. Proteom. 3 (2007) 13–21.
[38] M. Culea, N. Palibroda, P. Chereches-Panta, M. Nanulescu, Comparison of isotopic dilution methods for determination of theophylline in the plasma and saliva of infants and children, Chromatographia 53 (2001) S387–S389.
[39] B.M. Ham, J.T. Jacob, R.B. Cole, Single eye analysis and contralateral eye comparison of tear proteins in normal and dry eye model rabbits by MALDI-ToF mass spectrometry using wax-coated target plates, Anal. Bioanal. Chem. 387 (2007) 889–900.
[40] P. Nirde, M.L. Dereggi, G. Tsoupras, G. Torpier, P. Fressancourt, A. Capron, Excretion of ecdysteroids by schistosomes as a marker of parasite infection, FEBS Lett. 168 (1984) 235–240.
[41] N.P. Robertson, G.D. Cain, Tris(hydroxymethyl)aminomethane—an artifact detected as an aminotetrose by gas-chromatography mass-spectrometry, Anal. Biochem. 146 (1985) 103–107.