



Laboratory detection of strongyloidiasis: IgG-, IgG₄- and IgE-ELISAs and cross-reactivity with lymphatic filariasis

A. NORSYAHIDA,¹ M. RIAZI,² S. M. SADJJADI,³ Y. MUHAMMAD HAFIZNUR,¹ H. C. LOW,⁴ M. ZEEHAIDA⁵ & R. NOORDIN¹

¹Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden, Penang, Malaysia, ²School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia, ³Shiraz University of Medical Sciences, Shiraz, Iran, ⁴School of Mathematical Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia, ⁵Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia

SUMMARY

Enzyme-linked immunosorbent assays (ELISAs) were developed for the detection of IgG, IgG₄ and IgE antibodies against *Strongyloides stercoralis*. A commercial ELISA (IVD Research, USA) was also used, and the sensitivities and specificities of the four assays were determined. Serum samples from 26 patients with *S. stercoralis* infection and 55 patients with other infections or no infection were analysed. Sensitivities of the IgG₄, IgG, IgE and IgG (IVD) assays were 76.9%, 84.6%, 7.7% and 84.6%, respectively, while the specificities were 92.7%, 81.8%, 100% and 83.6%, respectively. If filariasis samples were excluded, the specificities of the IgG₄-ELISA and both IgG-ELISAs increased to 100% and 98%, respectively. A significant positive correlation was observed between IgG- and IgG₄-ELISAs ($r = 0.4828$; $P = 0.0125$). IgG- and IgG- (IVD) ELISAs ($r = 0.309$) were positively correlated, but was not significant ($P = 0.124$). Meanwhile there was no correlation between IgG₄- and IgG- (IVD) ELISAs ($r = 0.0042$; $P = 0.8294$). Sera from brugian filariasis patients showed weak, positive correlation between the titres of antifilarial IgG₄ and the optical densities of anti-*Strongyloides* IgG₄-ELISA ($r = 0.4544$, $P = 0.0294$). In conclusion, the detection of both anti-*Strongyloides* IgG₄ and IgG antibodies could improve the serodiagnosis of human strongyloidiasis. Furthermore, patients from lymphatic filariasis endemic areas who are serologically diagnosed with strongyloidiasis should also be tested for filariasis.

Keywords cross-reactivity, ELISA, filariasis, IgE, IgG, IgG₄, *Strongyloides stercoralis*, strongyloidiasis

INTRODUCTION

Strongyloidiasis poses a significant health threat to humans, as approximately 30–100 million people are infected worldwide, mostly in tropical and subtropical countries (1,2). It is well known that *Strongyloides stercoralis* is one of the most difficult parasitic infections to diagnose due to the difficulty of larval detection in human faeces, and a majority of strongyloidiasis cases involve asymptomatic chronic infections. To decrease the likelihood of disease progression to the devastating and often fatal *Strongyloides* hyperinfection syndrome, the identification and treatment of chronic intestinal strongyloidiasis are important, especially in immunocompromised individuals (3). Furthermore, untreated infected individuals may risk the occurrence of long-term persistent *Strongyloides* infections due to the ability of the parasite to replicate within the host by autoinfection.

The absence of a gold standard test for the diagnosis of strongyloidiasis has worsened the situation; therefore, numerous techniques have been developed to improve the sensitivities and specificities of the available detection methods. These methods are either traditional parasitological methods (i.e. faecal direct smear, formalin–ethyl acetate concentration, agar plate culture, Baermann concentration, Harada-Mori filter paper culture, and Kato-Katz thick smear) or serological methods [e.g. enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent test (IFAT)], which are useful complements to parasitological diagnoses of strongyloidiasis (4–6). The immunodiagnosis of active or recent *S. stercoralis* infections by ELISA has been reported to have

Correspondence: Rahmah Noordin, Ph.D., Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Penang, Malaysia (e-mail: rahmah8485@gmail.com).

Disclosures: None.

Received: 26 October 2012

Accepted for publication: 15 February 2013

approximately 85–95% sensitivity (7). However, these assays show variable sensitivity and specificity, depending on antigen preparation, immunoglobulin isotypes and the tested populations (8).

Current guidelines from the Infectious Diseases Society of America, the American Society of Transplantation, the Centers for Disease Control and Prevention and the American Society of Blood and Marrow Transplantation recommend *Strongyloides* IgG-ELISA testing of patients from endemic areas or of patients with gastrointestinal symptoms or eosinophilia prior to solid organ transplantation or hematopoietic stem cell (including autologous) transplantation (3,9,10). However, IgG₄ antibodies have been generally considered to be far more specific for the detection of intestinal helminth infections compared to IgG antibodies, which commonly cross-react with filarial antigens (11–13). In this regard, Muck and colleagues (13) recommend that diagnostic laboratories that use assays based on crude filarial lysates should rule out *Strongyloides* infections when positive antifilarial antibody responses are obtained.

In an effort to improve the serodiagnosis of human strongyloidiasis, this study was performed to evaluate the sensitivities and specificities of IgG-, IgG₄- and IgE-ELISAs using laboratory-based ELISAs and a commercial IgG-ELISA (IVD Research, Inc., Carlsbad, CA, USA).

MATERIALS AND METHODS

Patients and serum samples

A total of 26 serum samples were obtained from patients with parasitologically proven *Strongyloides* infection, and 55 sera were obtained from patients with other infections or with no infections (healthy controls) (Table 1). The samples were obtained from the serum banks of the institutions involved in this study and were previously collected according to the ethical requirements of each organization.

Table 1 List of serum samples used in this study

Diagnosis	No. of patients
Strongyloidiasis	26
Ancylostomiasis	2
Ascariasis	3
Amoebiasis	6
Giardiasis	2
Filariasis	23
Trichuriasis	3
Trichostrongyliasis	11
Healthy blood donor	5

Detection of *S. stercoralis* larvae in stool samples

Direct microscopic examination, using a normal saline (0.9% NaCl) and iodine wet smear, was performed for each stool sample. At least two slides were prepared from each stool sample, and more than 30 fields were examined per slide.

Antigen preparation

Lyophilized *S. stercoralis* filariform larvae were resuspended in 1 mL of 0.01 M phosphate-buffered saline (PBS), pH 7.2 that contained a cocktail of protease inhibitors (Roche Diagnostics, Mannheim, Germany), followed by incubation on ice for 10 min. The mixture was then frozen and thawed repeatedly by transfer between a liquid nitrogen tank and a 37°C water bath, respectively, followed by the addition of lysozyme at a final concentration of 0.5 mg/mL and subsequent incubation on ice for 10 min. The larvae were further disrupted using a sonicator, for five cycles at 30 s/cycle and a power of 1.5 Hz. The suspension was centrifuged at 10,000 × g for 10 min at 4°C, and the supernatant was analysed for protein content using an RCDC assay (Bio-Rad, Hercules, CA, USA) and then stored at –80°C. The leftover pellet was stirred in PBS overnight at 4°C to further extract the antigen and centrifuged at 10,000 × g for 10 min, and the protein content of the supernatant was determined as described above. Recombinant BmR1 antigen was previously produced in our laboratory according to a previously published method (14,15).

Laboratory-based ELISAs for the detection of anti-*Strongyloides* IgG, IgG₄ and IgE

Preliminary experiments were performed to determine the optimal conditions for ELISA, particularly antigen concentrations and dilutions of serum and secondary antibody conjugates. High-binding microtitre plates (Nunc MaxiSorp; Nalge Nunc International, Rochester, NY) were coated with 5 µg/mL of *S. stercoralis* antigen in 0.06 M carbonate buffer (pH 9.6) for IgG-ELISA, or 10 µg/mL of antigen for IgG₄ and IgE-ELISA, and were incubated overnight at 4°C. After five washes with 0.05% Tween-20 in PBS, the wells were blocked with 3% (w/v) bovine serum albumin (Sigma Aldrich Co, St. Louis, MO, USA) in PBS for 1 h at 37°C. Subsequent steps were carried out using PBS as the diluent, and washes with PBS-T were performed on a plate shaker (500 rpm) between the incubation steps. Serum samples were diluted at 1 : 100 for IgG₄- and IgE-ELISAs, and 1 : 200 for IgG-ELISAs. After incubating the serum samples for 2 h at 37°C on a

microplate shaker (300 rpm), the plates were washed as described above. The secondary antibody conjugates were added for 30 min at 37°C (1 : 4500 for IgG₄-HRP, 1 : 2000 for IgE-HRP and 1 : 8000 for IgG-HRP), followed by an incubation with ABTS substrate solution (Roche Diagnostics). The absorbance readings of the reactions were read at 405 nm, using 490 nm readings as a reference, on a Thermo Multiskan Spectrum Reader (Multiskan Spectrum, Thermo Scientific, Rockford, IL, USA). The results were expressed as an ELISA index (EI), which was calculated by dividing the absorbance of the test sample (the optical density at 450 nm wavelength or OD_{405 nm}) by the cut-off value (COV). The COV for each of the laboratory ELISAs was calculated based on the Student *t*-distribution of the negative control sera readings, following the equation by Dixon and Massey, 1983 (16,17). The equation used for the COV calculation is as follows:

$$\text{Cut-off} = X + SDf \quad (1)$$

In this equation, *X* is the mean of independent control sera readings, *SD* is the standard deviation, and *f* is the standard deviation multiplier which is derived from the critical values for a one-tailed *t*-distribution (refer to Federighi (18) for a comprehensive listing of critical values for a *t*-distribution).

Laboratory-based ELISA for the determination of antifilarial IgG₄ antibody

The antifilarial IgG₄-ELISA was performed as above with a few modifications, using sera from brugian filariasis patients. BmR1 filarial recombinant antigen (20 µg/mL) was used to coat the microtitre plate. The secondary antibody, antihuman IgG₄-HRP, was diluted to 1 : 4500. Serial dilutions of the serum samples were made from 1 : 200 to 1 : 25 600 in PBS, pH 7.2.

Commercially based ELISA

The *Strongyloides* Serology Microwell ELISA (IVD Research, Inc., Carlsbad, CA, USA), which is based on the detection of human IgG antibodies against *Strongyloides spp.* antigen, was performed according to the manufacturers instructions. In brief, serum samples were diluted 1 : 64 in dilution buffer and incubated for 10 min in the antigen-coated wells. After three washes with wash buffer, two drops of conjugate solution were added and incubated for 5 min. Subsequently, the wells were washed again as described above followed by the addition of two drops of chromogenic solution. Following a 5-min incubation, the reaction was stopped with two drops of stop solution, and

the results were immediately read at 450 nm (reference: 620 nm). The COV given for this test is 0.200.

Statistical analyses

The statistical significance of the difference in *S. stercoralis*-specific antibody titres was analysed by one-way ANOVA test. Pearson correlation coefficient (*r*) test was used to analyse the correlations among the levels of IgG and IgG₄, IgG and IgG (IVD) and IgG₄ and IgG (IVD) antibodies to *Strongyloides spp.* Spearman correlation test was used to analyse correlation between the anti-*Strongyloides* IgG₄ (OD₄₀₅) results and the antifilarial IgG₄ antibody titres in filariasis serum. Statistical tests were performed using GraphPad Prism version 5 (San Diego, CA, USA). In addition, a paired *t*-test was used to determine whether the difference in the specificities of the two IgG-ELISAs was significant. In all cases, differences were considered as statistically significant when *P* < 0.05.

RESULTS AND DISCUSSION

In this study, we examined parasite-specific IgG₄, IgG and IgE responses against *S. stercoralis* infection using laboratory ELISAs as well as a commercial IgG-ELISA (IVD). The COVs and results obtained for all ELISAs are shown in Table 2. Of the 26 patients who were faecally positive for *Strongyloides*, 20 were seropositive for specific IgG₄ antibody with a sensitivity rate of 76.9%, 22 (84.6%) were seropositive by both laboratory and commercial (IVD) IgG tests, and only 2 were seropositive for parasite-specific IgE antibody (7.7%). Further studies using much larger sample size will need to be performed to confirm the results of this small scale study. These preliminary findings would be a useful guide in designing the larger study.

Several studies have addressed the importance of immunoglobulin isotypes in immune responses to helminths. Parasite-specific IgG has been reported to be important

Table 2 Comparison between sensitivity and specificity of the detection of *Strongyloides stercoralis* IgG₄, IgG and IgE antibodies by lab-based ELISA and commercial ELISA kit (IVD)

Results	Lab-based ELISA			Commercial ELISA kit (IVD)
	IgG ₄ COV: 0.21	IgG COV: 0.34	IgE COV: 0.2	IgG COV: 0.2
Sensitivity <i>n</i> = 26	76.9	84.6	7.7	84.6
Specificity <i>n</i> = 55	92.7	81.8	100	83.6

during the initial invasive phase, irrespective of the immune status (19). A prominent IgG₄ response has been observed in chronically infected strongyloidiasis patients, as well as in patients with other helminth infections, such as filariasis (20–24). Furthermore, the IgG₄ response was reported to be up-regulated early and to persist in chronic infections (21,25), while IgE levels were reported to be down-regulated as the duration of infection increased (25,26). Other investigators have reported that IgG₄ may block IgE-mediated immune responses (19), as described in Atkins *et al.* (21). Because the prevalence of IgG₄ among the patients in this study was quite high, the IgG₄ effect may explain the low prevalence of parasite-specific IgE. Unfortunately, clinical and historical data from the infected patients in this study were not available; therefore, any speculation regarding a correlation of the serological results with clinical manifestation, infection chronicity, age and gender could not be made.

Figure 1 shows the levels of parasite-specific IgG₄, IgG and IgE antibodies to *S. stercoralis* in the positive serum samples. An analysis of variance showed significant increases in the detection sensitivities of both IgG tests (i.e. laboratory and commercial [IVD] ELISAs) compared to the IgG₄-ELISA ($P = 0.0028$ and $P = 0.0446$, respectively). Thus, this study showed that IgG₄ is less sensitive than IgG in detecting strongyloidiasis. There was no significant difference between the results of the laboratory and commercial (IVD) IgG-ELISAs ($P = 0.5045$); this may be due to the detection of the same antibody (IgG) and the use of *Strongyloides* larval lysate antigen in both assays.

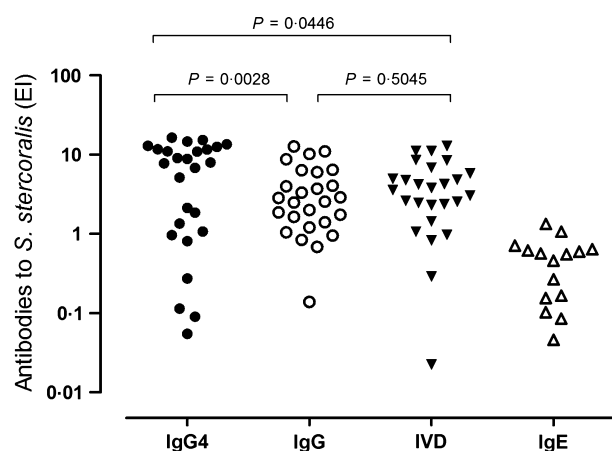


Figure 1 Levels of parasite-specific IgG₄, IgG, IgE (by lab-based ELISA) and IgG (by commercial ELISA kit IVD) antibodies to *Strongyloides stercoralis* expressed in ELISA index measured in 26 serum samples of patients with strongyloidiasis.

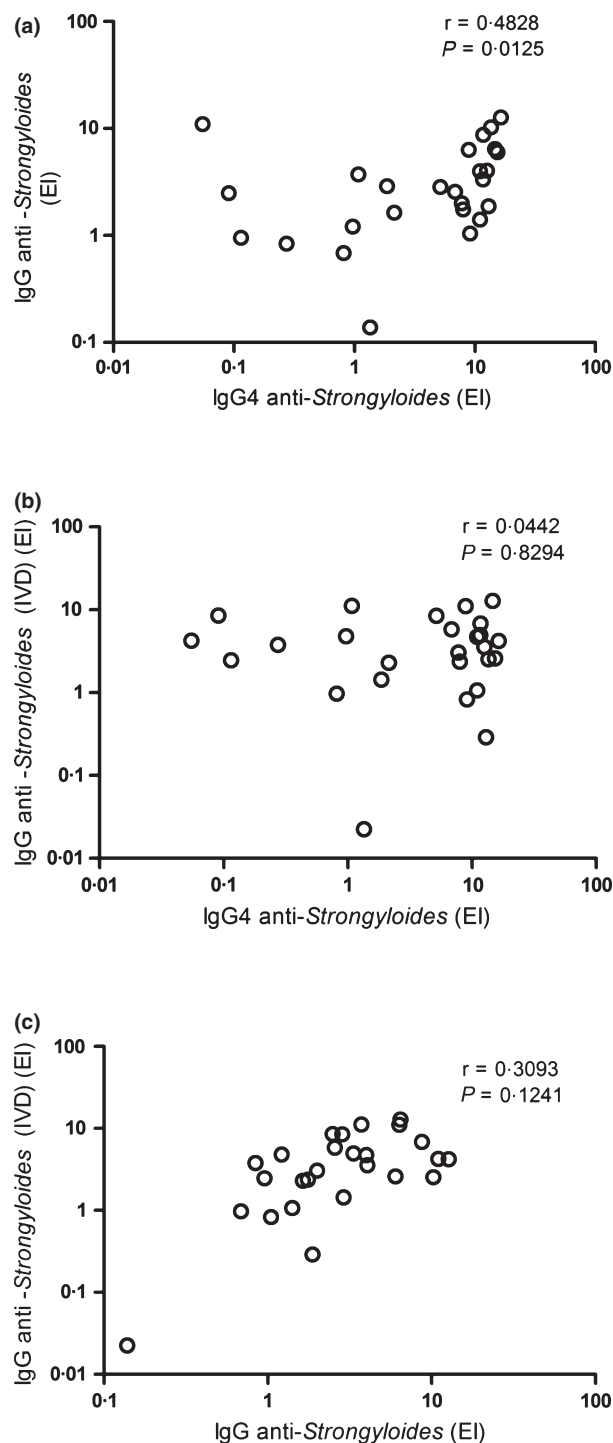


Figure 2 Correlation between levels of specific IgG (lab-based) and IgG₄ (a), IgG₄ and IgG (IVD) (b), IgG (lab-based) and IgG (IVD) (c) antibodies to *Strongyloides stercoralis* in positive serum samples of patients with strongyloidiasis. Coefficients of correlation were determined by the Pearson correlation test. Differences were considered as statistically significant when $P < 0.05$.

A significant positive correlation was observed between levels of specific IgG- and IgG₄ ($r = 0.4828$; $P = 0.0125$; Figure 2a); and no correlation observed between IgG₄- and IgG- (IVD) ($r = 0.0042$; $P = 0.8294$; Figure 2b). Meanwhile comparison between IgG- and IgG- (IVD) ($r = 0.309$) showed weak correlation; however, it was not significant ($P = 0.124$; Figure 2c). Although the two IgG tests used *Strongyloides* lysate antigen, the parasite species and methods of lysate preparation are not exactly the same, this may explain the nonsignificant correlation between the two tests.

Of the 55 serum samples from patients with various other parasitological infections or no infections, anti-*Strongyloides* IgG₄ antibody was detected in four filariasis patients, giving a specificity rate of 92.7%, while IgG was detected in 10 subjects (9 filariasis and 1 trichostrongyliasis patients) by laboratory-based ELISA (81.8% specificity) and 9 subjects (eight filariasis and one trichostrongyliasis patients) by commercial (IVD) ELISA (83.6% specificity).

Paired *t*-test analysis showed no significant difference between the specificities of the two IgG-ELISAs ($P = 0.202$). By excluding the results of the filariasis samples, the specificities of the IgG₄- ELISA and both of the IgG-ELISAs increased to 100% and 98%, respectively. Thus, although the IgG₄-ELISA is less sensitive than the IgG-ELISAs, the former is more specific.

To determine whether the cross-reactivity with filariasis patient sera was influenced by the abundance of antifilarial antibodies, titrations of IgG₄ were performed on the filariasis patient serum samples, followed by an analysis of the correlation with the results of the *Strongyloides* IgG₄-ELISA (Figure 3). The two parameters were found to be weakly correlated (Spearman rho = 0.4544; $P = 0.0294$). Although previous investigators had reported cross-reactivity between strongyloidiasis and filariasis (4,13,27), this study demonstrated that the binding of the *Strongyloides* antigen to the antifilarial antibodies was not much influenced by the titre of the latter. It is thus highly recommended that, in filariasis endemic area, positive serological cases of strongyloidiasis should also be tested for filariasis before confirming the serodiagnosis. For brugian filariasis, a commercially available test called Brugia Rapid (Reszon

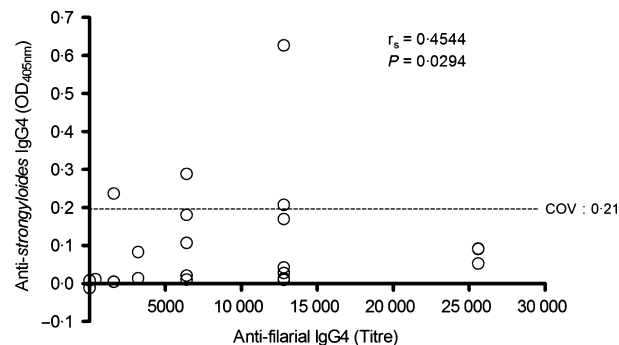


Figure 3 Correlation studies between optical density values of anti-*Strongyloides* specific IgG₄ (lab-based) and the titer of anti-filarial specific IgG₄ (lab-based) in positive serum samples of patients with filariasis ($n = 23$). Coefficients of correlation were determined by the Spearman correlation test and the differences were considered as statistically significant.

Diagnostics International Sdn. Bhd., Selangor, Malaysia) can be used to assist with this differential diagnosis because the test has been shown to be highly specific (>95%) when tested with serum samples from patients with strongyloidiasis (28,29). In this regard, a 31-kDa *Strongyloides* recombinant antigen (NIE) has been reported to be specific against antibodies to nonlymphatic and lymphatic filariasis (27,30,31) and thus is potentially useful as a diagnostic reagent.

In conclusion, because the detection of parasite-specific IgG₄ antibodies is more specific but less sensitive than the detection of parasite-specific IgG antibodies, the combined use of IgG and IgG₄ assays would be helpful in improving the serodiagnosis of strongyloidiasis. Efforts to develop field-applicable rapid tests using recombinant antigen(s) that do not cross-react with antibodies to lymphatic and nonlymphatic filaria should be encouraged.

ACKNOWLEDGEMENTS

This study was funded by Universiti Sains Malaysia Research University grant, No: 1001/CIPPM/812078 and USM short-term grant No. 304/PPSP/61312089. We gratefully acknowledge the contributions of Madihah Basuni and Dr Khoo Boon Yin in this study.

REFERENCES

- Bethony J, Brooker S, Albonico M, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 2006; **367**: 1521–1532.
- Kramme S, Nissen N, Soblik H, et al. Novel real-time PCR for the universal detection of *Strongyloides* species. *J Med Microbiol* 2011; **60**: 454–458.
- Roxby CA, Gottlieb SG & Limaye PA. Strongyloidiasis in transplant patients. *Clin Infect Dis* 2009; **49**: 1411–1423.
- Conway DJ, Atkins NS, Lillywhite JE, et al. Immunodiagnosis of *Strongyloides stercoralis* infection: a method for increasing the specificity of the indirect ELISA. *Trans R Soc Trop Med Hyg* 1993; **87**: 173–176.
- Boscolo M, Gobbo M, Mantovani W, et al. Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clin Vaccine Immunol* 2007; **14**: 129–133.
- Silva LP, Barcelos IS, Passos-Lima AB, et al. Western blotting using *Strongyloides ratti* antigen for the detection of IgG antibodies as

- confirmatory test in human strongyloidiasis. *Mem Inst Oswaldo Cruz* 2003; **98**: 687–691.
- 7 Ravi V, Ramachandran S, Thompson RW, Andersen JF & Neva FA. Characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. *Mol Biochem Parasitol* 2002; **125**: 73–81.
 - 8 Olsen A, van Lieshout L, Marti H, *et al.* Strongyloidiasis – the most neglected of the neglected tropical disease? *Trans R Soc Trop Med Hyg* 2009; **103**: 967–972.
 - 9 Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients recommendations of CDC, the Infectious Disease Society of America, and the American Society of Blood and Marrow Transplantation. *MMWR Recomm Rep* 2000; **49**: 1–125.
 - 10 Screening of donor and recipient prior to solid organ transplantation. *Am J Transplant* 2004; **4**(Suppl 10): 10–20.
 - 11 Lal RB & Ottesen EA. Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. *J Infect Dis* 1988; **158**: 1034–1037.
 - 12 Eberhard ML & Lammie PJ. Filariasis. *Clin Lab Med* 1991; **11**: 977–1010.
 - 13 Muck AE, Pires ML & Lammie PJ. Influence of infection with non-filarial helminths on the specificity of serological assays for anti-filarial immunoglobulin G4. *Trans R Soc Trop Med Hyg* 2003; **97**: 88–90.
 - 14 Rahmah N, Lim BH, Azian H, Ramelah TS & Rohana AR. Short communication: use of a recombinant antigen-based ELISA to determine prevalence of brugian filariasis among Malaysian schoolchildren near Pasir Mas, Kelantan-Thailand border. *Trop Med Int Health* 2003; **8**: 158–163.
 - 15 Norsyahida A, Madihah B, Chew AL, Ahmad Ramli MY & Rahmah N. Purification of *BmR1* recombinant antigen. *Protein J* 2010; **29**: 509–515.
 - 16 Dixon WJ & Massey FJ. In *Introduction to Statistical Analysis*, 4th edn. New York, McGraw-Hill, 1983: 92.
 - 17 Frey A, di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. *J Immunol Methods* 1998; **221**: 35–41.
 - 18 Federighi ET. Extended tables of the percentage points of students *t*-distribution. *J Am Stat Assoc* 1959; **54**: 683–688.
 - 19 Marcos LA, Terashima A, Canales M & Gotuzzo E. Update on strongyloidiasis in the immunocompromised host. *Curr Infect Dis Rep* 2011; **13**: 35–46.
 - 20 Genta RM & Lillibridge JP. Prominence of IgG4 antibodies in the human responses to *Strongyloides stercoralis* infection. *J Infect Dis* 1989; **160**: 692–699.
 - 21 Atkins NS, Conway DJ, Lindo JF, *et al.* L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. *Parasite Immunol* 1999; **21**: 517–526.
 - 22 Satoh M, Toma H, Sato Y, *et al.* Production of high level of specific IgG4 antibody associated with resistance to albendazole treatment in *HLA-DRB1*0901*-positive patients with strongyloidiasis. *Am J Trop Med Hyg* 1999; **61**: 668–671.
 - 23 Rodrigues RM, de Oliveira MC, Sopenete MC, *et al.* IgG1, IgG4, and IgE antibody responses in human strongyloidiasis by ELISA using *Strongyloides ratti* saline extract as heterologous antigen. *Parasitol Res* 2007; **101**: 1209–1214.
 - 24 Supali T, Rahmah N, Djuardi Y, Sartono E, Ruckert P & Fischer P. Detection of filarial-specific IgG4 antibodies using *Brugia Rapid* test in individuals from an area highly endemic for *Brugia timori*. *Acta Trop* 2004; **90**: 255–261.
 - 25 Atkins NS, Lindo JF, Lee MG, *et al.* Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Trans R Soc Trop Med Hyg* 1997; **91**: 609–613.
 - 26 Gill GV, Bell DR & Fifield R. Lack of immunoglobulin E response to longstanding strongyloidiasis. *Clin Exp Immunol* 1979; **37**: 292–294.
 - 27 Ramanathan R, Burbelo PD, Groot S, *et al.* A luciferase immunoprecipitation systems assay enhances the sensitivity and specificity of diagnosis of *Strongyloides stercoralis* infection. *J Infect Dis* 2008; **198**: 444–451.
 - 28 Rahmah N, Taniawati S, Shenoy RK, *et al.* Specificity and sensitivity of a rapid dipstick test (*Brugia Rapid*) in the detection of *Brugia malayi* infection. *Trans R Soc Trop Med Hyg* 2001; **95**: 601–604.
 - 29 Rahmah N, Shenoy RK, Nutman TB, *et al.* Multicentre laboratory evaluation of *Brugia Rapid* dipstick test for detection of brugian filariasis. *Trop Med Int Health* 2003; **8**: 895–900.
 - 30 Ramachandran S, Thompson RW, Gam AA & Neva FA. Recombinant cDNA clones for immunodiagnosis of strongyloidiasis. *J Infect Dis* 1998; **177**: 196–203.
 - 31 Requena-Mendez A, Chiodini P, Bisoffi Z, *et al.* The laboratory diagnosis and follow up of strongyloidiasis: a systemic review. *PLoS Negl Trop Dis* 2013; **7**: e2002.