UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

PERBANDINGAN ANTIGEN LARUT (SA) BRUGIA MALAYI DAN BRUGIA PAHANGI DI DALAM PENGEMBANGAN SISTEM ELISA UNTUK DIAGNOSIS PENYAKIT FILARIA

PENYELIDIK

DR. RAHMAH NOORDIN

PENYELIDIK BERSAMA

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BAHAGIAN PENYELIDIKAN & PEMBANGUNAN

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Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** ....DR..RAHMAH.NOORDIN.....

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Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* : ..PROF..KHAIRUL.ANUAR.ABDULLAH.....

DR..AFIFI.SHEIKH.ABU.BAKAR.....

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2) **Pusat Pengajian/Pusat/Unit** : Pusat Pengajian Sains Perubatan.....

....Kubang.Kerian,..Kelantan.....

3) **Tajuk Projek:** Perbandingan Antigen Larut (SA) Brugia malayi dan.....

Brugia.pahangi.di.dalam.pengembangan.sistem.ELISA.untuk.diagnosis.....

penyakit Filaria

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4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

Sila lihat lampiran 1(a) dan (b)

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Bahasa ~~Malaysia~~ (Inggeris)	**Bahasa ~~Inggeris~~** (Malaysia)
Brugia malayi	Brugia malayi
Brugia pahangi	Brugia pahangi
Soluble antigen	Antigen larut
IgG 2 - ELISA	IgG 2 - ELISA
IgG 4 - ELISA	IgG 4 - ELISA
immunodiagnosis	pendiagnosan imun
brugian filariasis	penyakit filaria brugian

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

"Brugia pahangi adult worm antigen is an unsuitable (substitute) for Brugia malayi adult worm antigen in the immunodiagnosis of brugian filariasis"
- Telah diterima untuk penerbitan di dalam jurnal Tropical Biomedicine
(salinan kertas kerja dilampirkan)

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

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(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah -

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ii) Pelajar Prasiswazah: -

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iii) Lain-Lain : -

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6. **Peralatan Yang Telah Dibeli:**

1. Multipipette .....
2. Adapter for multipipette .....

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UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

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untuk pengesahan &
tandatangan Dekan

b/p DATO' PROFESOR MUSTAFFA EMBONG
DEKAN/PROFESOR PERUBATAN
PUSAT PENGAJIAN SAINS PERUBATAN
UNIVERSITI SAINS MALAYSIA
16150 KUBANG KERIAN
KELANTAN.

Lampiran 1a

Antigen cacing dewasa *Brugia pahangi* tidak sesuai menjadi pengganti antigen cacing dewasa *Brugia malayi* di dalam pendiagnosan imun penyakit filaria brugian

Abstrak. Antigen larut cacing dewasa *Brugia malayi* dan *Brugia pahangi* telah digunakan di dalam 'digoxigenin-sandwich ELISA' untuk mengesan antibodi-antibodi anti-filarial IgG4 dan anti-filarial IgG2 dalam beberapa kumpulan pesakit filaria, pesakit yang dijangkiti helmin bawaan tanah dan manusia normal. Kedua-dua jenis antigen menunjukkan tindakbalas IgG4 yang positif dengan semua serum dari kumpulan bermikrofilaria. Tetapi ELISA yang menggunakan antigen *B. pahangi* juga menunjukkan keputusan ELISA yang positif dengan sesetengah serum pesakit helmin bawaan tanah dan sesetengah serum manusia normal. Sebaliknya, tidak ada tindakbalas silang yang dipamerkan apabila *B. malayi* digunakan sebagai antigen. Di dalam analisa tindakbalas anti-filarial IgG2, semua serum dari kumpulan pesakit untut yang kronik menunjukkan keputusan ELISA yang positif dengan penggunaan *B. malayi* sebagai antigen; tidak terdapat tindakbalas silang dengan serum dari pesakit helmin yang lain. Ini dibandingkan dengan keputusan IgG2-ELISA yang mengunakan antigen *B. pahangi*; yang mana hanya sebahagian pesakit untut yang memberi keputusan yang positif. Selain dari itu, dengan penggunaan antigen *B. pahangi* , sesetengah serum dari pesakit helmin bawaan tanah dan individu normal juga mempamerkan keputusan yang positif. Dengan itu, kajian ini membuktikan yang antigen larut cacing dewasa *Brugia pahangi* tidak sesuai digunakan sebagai pengganti untuk antigen larut cacing dewasa *Brugia malayi* di dalam pendiagnosan penyakit filaria brugian melalui kaedah ELISA.

Lampiran 1b

Brugia pahangi adult worm antigen is an unsuitable substitute for _Brugia malayi_ adult worm antigen in the immunodiagnosis of brugian filariasis

Abstract. _Brugia malayi_ and _Brugia pahangi_ adult worm soluble antigens were used in digoxigenin-sandwich ELISA to detect filarial specific anti-IgG4 and anti-IgG2 antibodies in different groups of filarial patients, soil-transmitted helminth infected patients and in normal individuals. Both kinds of antigens showed positive IgG4 responses with all sera of microfilaraemic patients. However, ELISA using _B. pahangi_ antigen also showed positive ELISA readings with some sera of patients with soil-transmitted helminth infections and some sera of normal individuals. On the other hand no such cross-reactivities were demonstrated when _B. malayi_ was used as the antigen. In the analysis of anti-filarial IgG2 antibody responses, all chronic elephantiasis sera gave positive readings in ELISAs that employed _B. malayi_ as the antigen; and no cross-reactivity with other helminthic infections were observed. In comparison, only some sera of chronic elephantiasis patients were positive for anti-filaral IgG2 antibodies when _B. pahangi_ was used as the antigen. Furthermore, with the use of _B. pahangi_ antigen coated plates, some sera from soil-transmitted helminth infected patients and from normal individuals also showed positive titres of anti-filarial IgG2 antibodies. This study demonstrated that _B. pahangi_ soluble adult worm antigen is not a suitable substitute for _B. malayi_ soluble adult worm antigen in the diagnosis of brugian filariasis by ELISA.

Research Note

Brugia pahangi adult worm antigen is an unsuitable substitute for *Brugia malayi* adult worm antigen in the immunodiagnosis of brugian filariasis

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Abstract. *Brugia malayi* and *Brugia pahangi* adult worm soluble antigens were used in digoxigenin-sandwich ELISA to detect filarial specific anti-IgG4 and anti-IgG2 antibodies in different groups of filarial patients, soil-transmitted helminth infected patients and in normal individuals. Both kinds of antigens showed positive IgG4 responses with all sera of microfilaraemic patients. However, ELISA using *B. pahangi* antigen also showed positive ELISA readings with some sera of patients with soil-transmitted helminth infections and some sera of normal individuals. On the other hand no such cross-reactivities were demonstrated when *B. malayi* was used as the antigen. In the analysis of anti-filarial IgG2 antibody responses, all chronic elephantiasis sera gave positive readings in ELISAs that employed *B. malayi* as the antigen; and no cross-reactivity with other helminthic infections were observed. In comparison, only some sera of chronic elephantiasis patients were positive for anti-filaral IgG2 antibodies when *B. pahangi* was used as the antigen. Furthermore, with the use of *B. pahangi* antigen coated plates, some sera from soil-transmitted helminth infected patients and from normal individuals also showed positive titres of anti-filarial IgG2 antibodies. This study demonstrated that *B. pahangi* soluble adult worm antigen is not a suitable substitute for *B. malayi* soluble adult worm antigen in the diagnosis of brugian filariasis by ELISA.

The procedure for the ELISA was as previously described (Abdul Wahab *et al.*, 1994). Based on initial optimization experiments, filarial antigen containing 10 µg/ml of protein was coated at 4^0C overnight in polyvinyl microtitre plates (Falcon, USA). Blocking was done with 0.5 % bovine serum albumin in PBS and washing steps were performed using PBS containing 0.05% Tween 20. Serum specimens were diluted 1: 50 and incubated for 2 h at room temperature. After a washing step, 1:1000 dilution of anti-IgG2 or anti-IgG4 conjugated to digoxigenin (DIG; Boehringer Mannheim, Germany) were incubated for 2 h. This was followed by incubation with anti-DIG-peroxidase (Boehringer Mannheim, Germany) at 1:1000 for a further 2 h before the addition of the chromogenic substrate (H_2O_2 and ortho-phenyldiamine).

The results are presented in Tables 1a and 1b. Based on the previous related study (Rahmah *et al.*, 1994), optical densities of greater than 0.250 were considered as positive results. Both *Brugia malayi* and *Brugia pahangi* antigens showed positive anti-filarial IgG4 responses with sera from all microfilaraemic patients. However, ELISA using *B. pahangi* antigen also showed positive ELISA readings with one out of 5 sera of each of *Ascaris lumbricoides, Trichuris trichiura* and mixed infection patients. In addition two out of 20 normal sera from endemic area and 1 out of 10 sera from city dwellers gave positive ELISA titres in assays utilizing *B. pahangi* antigen. On the other hand, IgG4-ELISA using *B. malayi* antigen did not demonstrate any cross-reactivity.

In the analysis of anti-filarial IgG2 antibodies, all chronic elephantiasis sera gave positive readings in ELISAs that employed *B. malayi* as the antigen; and no cross-reactivity with other helminthic infections were observed. However, in ELISAs using *B. pahangi* as antigen, only 2 out of 5 sera of chronic elephantiasis patients gave positive optical densities. In addition, one out of 5 sera of each of *Ascaris lumbricoides* and *Trichuris trichiura* patients gave positive optical density readings. Furthermore, there were also false positive readings shown by normal sera from endemic area and city dwellers.

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Jirds (*Meriones unguiculatus*) infected with *B. pahangi* developed lymphatic lesions that histologically resembled the human filarial lesions, caused by *B. malayi* and *W. bancrofti* , in which dilation of lymphatic vessels, fibrosis and granulamatous inflammation occurred (Ah & Thompson, 1973; Vincent *et al.*, 1980). These and other studies (Spencer *et al.*, 1981; Maizels *et al.*, 1983) have shown the close relationships between *B. pahangi* and *B. malayi*. However, successful filarial infection of laboratory animals (e.g. jirds and cats) have been shown to be more readily achieved when using *B. pahangi* rather than *B. malayi* infective larvae (Wilson & Ramachandran 1971; Ash, 1973; Denham, 1974). Being an animal filarial parasite, it is also much safer to breed mosquitoes infected with *B. pahangi* in the insectorium than to breed *B. malayi* - infected mosquitoes. Therefore it would be advantagous if *B. malayi* could be substituted with *B. pahangi* as the antigen source for studies in lymphatic filariasis.

In the previous study (Rahmah *et al.*, 1994), we reported that the detection of IgG, IgG1 and IgG3 antibodies in sera of brugian filariasis patients using ELISA was not specific. However the responses of IgG2 and IgG4 antibodies were specific in chronic elephantiasis sera and microfilaraemic sera respectively. These results were obtained by employing *B. malayi* antigen coated plates. In the present study, IgG2-ELISA and IgG4-ELISA were used to investigate the specificities of the tests when *B. pahangi* antigen was used in coating the microtitre plates.

The results of this study demonstrated that ELISAs that employed *B. pahangi* antigen showed cross-reactivities to other nematode infections and thus were not specific; while ELISAs that employed *B. malayi* antigen were specific. Thus, although *Brugia malayi* and *Brugia pahangi* are closely related organisms, there may exist significant differences in their antigenic epitopes. Western blot analysis of the antigenic epitopes of these two filarial species that are recognised by anti-filarial IgG2 and IgG4 antibodies will be performed to provide further understanding and insight into the observed differences in specificity. In conclusion, adult worm soluble

antigen of *B. pahangi* seemed to be an unsuitable substitute for *B. malayi* adult worm soluble antigen in the immunodiagnosis of brugian filariasis by ELISA.

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Legend to tables

Table 1(a). Results of sandwich DIG-ELISA using *Brugia malayi* and *Brugia pahangi* antigens to detect anti-filarial IgG4 antibodies in various categories of patients. Postive results are those with optical densities > 0.250.

Table 1(b). Results of sandwich DIG-ELISA using *Brugia malayi* and *Brugia pahangi* antigens to detect anti-filarial IgG2 antibodies in various categories of patients. Postive results are those with optical densities > 0.250.

Table 1 (a)

Sera Sample	No	IgG4-ELISA			
		Brugia malayi		*Brugia pahangi*	
		No. samples with O.D > 0.250 (mean ± SD)	No. samples with O.D < 0.250 (mean ± SD)	No. samples with O.D > 0.250 (mean ± SD)	No. samples with O.D < 0.250 (mean ± SD)
Total	63				
Microfilaraemic	6	6 (0.517 ± 0.042)	0	6 (0.313 ± 0.015)	0
Chronic elephantiasis	7	0	7 (0.083 ± 0.033)	0	7 (0.089 ± 0.045)
Ascaris lumbricoides positive	5	0	5 (0.050 ± 0.083)	1 (0.299)	4 (0.093 ± 0.055)
Trichuris trichiura positive	5	0	5 (0.042 ± 0.020)	1 (0.405)	4 (0.060 ± 0.046)
Hookworm positive	5	0	5 (0.033 ± 0.025)	0	5 (0.086 ± 0.028)
Mixed infection	5	0	5 (0.025 ± 0.020)	1 (0.287)	4 (0.043 ± 0.088)
Normal sera from endemic area	20	0	20 (0.050 ± 0.025)	2 (0.296 ± 0.020)	18 (0.056 ± 0.025)
Normal sera from city-dwellers	10	0	10 (0.067 ± 0.045)	1 (0.413)	9 (0.041 ± 0.035)

Table 1 (b)

Sera Sample	No	IgG2-ELISA			
		Brugia malayi		*Brugia pahangi*	
		No. samples with O.D > 0.250 (mean ± SD)	No. samples with O.D < 0.250 (mean ± SD)	No. samples with O.D > 0.250 (mean ± SD)	No. samples with O.D < 0.250 (mean ± SD)
Total	63				
Microfilaraemic	6	0	6 (0.145 ± 0.058)	0	6 (0.210 ± 0.013)
Chronic elephantiasis	7	7 (0.567 ± 0.017)	0	2 (0.466 ± 0.020)	5 (0.163 ± 0.040)
Ascaris lumbricoides positive	5	0	5 (0.075 ± 0.033)	1 (0.315)	4 (0.115 ± 0.055)
Trichuris trichiura positive	5	0	5 (0.117 ± 0.075)	1 (0.408)	4 (0.165 ± 0.033)
Hookworm positive	5	0	5 (0.152 ± 0.066)	0	5 (0.183 ± 0.028)
Mixed infection	5	0	5 (0.150 ± 0.099)	0	5 (0.194 ± 0.045)
Normal sera from endemic area	20	0	20 (0.142 ± 0.058)	2 (0.278 ± 0.015)	18 (0.159 ± 0.075)
Normal sera from city-dwellers	10	0	10 (0.150 ± 0.033)	1 (0.310)	9 (0.175 ± 0.025)