

SIZE AND CHARGE ANTIGENS OF *DIROFILARIA IMMITIS* ADULT WORM FOR IgG-ELISA DIAGNOSIS OF BANCROFTIAN FILARIASIS

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Abstract. We used *Dirofilaria immitis* adult worm antigens to develop an IgG-ELISA, then used this to evaluate 30 serum samples of patients with proven *Wuchereria bancrofti* infection, 131 samples of patients with other parasitic diseases and 30 serum samples of healthy controls. The *D. immitis* antigen was prepared using two methods: Sephacryl S-200 chromatography and iso-electric focusing with a Rotofor cell. This was done to determine the best method for diagnosing *W. bancrofti* filariasis. Before fractionation, crude male *D. immitis* antigen yielded 100% sensitivity and 60.8% specificity, and crude female antigen yielded 80% sensitivity and 52.8% specificity, respectively, to detect *W. bancrofti* infection. After gel filtration chromatography, the male *D. immitis* antigen, called MP1, yielded 100% sensitivity and 95% specificity, and female *D. immitis* antigen, called FmP1, gave 100% sensitivity and 59.6% specificity, to detect *W. bancrofti* infection. Using iso-electric-focusing, both male and female crude *D. immitis* antigens (Iso-MF and Iso-FmF, respectively) were separated mechanically into 20 iso-fractions (F1-F20) each. By preliminary screening with ELISA, using pooled positive and negative sera, Iso-MF10, pH 7.5, and Iso-FmF14, pH 7.6, were selected. Iso-MF10 gave 100% sensitivity and 96.9% specificity, and Iso-FmF14 gave 100% sensitivity and 64% specificity. In the study, Og4C3-ELISA, for the detection of circulating filarial antigen, was also used to analyze these serum samples, it gave 87.6% sensitivity and 99.4% specificity to detect *W. bancrofti* infection. Male *D. immitis* antigens, MP1 and Iso-MF10, gave high sensitivity and specificity, and appear to be the best choices for use in an ELISA to diagnose bancroftian filariasis.

Key words: *Dirofilaria* antigen, column chromatography, iso-electric focusing, IgG-ELISA, Og4C3-ELISA

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INTRODUCTION

In Thailand, *Wuchereria bancrofti* and *Brugia malayi* are prevalent in rural border areas, with an estimated 3 million people at risk (WHO, 2001). Bancroftian filariasis is endemic along the Thai-Myanmar

border, where an extremely high prevalence is seen among Myanmar immigrant workers (Triteeraprapab *et al*, 2000; Bhumiratana *et al*, 2005). Brugian filariasis is endemically limited to a few southern provinces in Thailand (Guptavani *et al*, 1977). New Thai and Myanmar patients have been found with bancroftian filariasis in sentinel and non-sentinel provinces. The mosquito vector, *Culex quinquefasciatus* Thai strain has been shown to transmit *W. bancrofti* Myanmar strain leading to infection (Triteeraprapab *et al*, 2000).

The different diagnostic tools for detection of human filariasis have been used in phase I of the Global Program to Eliminate Lymphatic Filariasis (GPELF). Antibody detection is the method used to detect bancroftian filariasis (Weil and Ramzy, 2006; WHO, 2006). With immunological tests it is not necessary to collect the samples at night. Although commercial test kits (Og4C3 and ICT) are used to detect the circulating filarial antigens of *W. bancroftian* (Turner *et al*, 1993; Weil *et al*, 1997) several studies have demonstrated problems with these methods (Rocha *et al*, 1996; 2009; Kimura *et al*, 2003). The definitive host of *W. bancrofti* is humans. Experimental animals have been used for raising the developmental stages of *W. bancrofti*, but these animals must be treated with immunosuppressive drugs. Therefore, the use of IgG antibodies against *D. immitis* to determine *W. bancrofti* infection would make the diagnosis and laboratory techniques easier to carry out. Adult *D. immitis* worms have been studied and tested in various laboratories (Smith *et al*, 1971; Grove *et al*, 1977; Frank and Grieve, 1996; Dekumyoy *et al*, 2000a).

Detection of antibodies using crude antigens often shows cross-reaction with other diseases, and occasionally, with healthy normal sera. To reduce cross-

reactivity and improve specificity, several fractionation/purification methods have been employed. Molecular sieve chromatography has been used to detect *Paragonimus heterotremus* antigens (Sephadex G-200) (Indrawati *et al*, 1991) and *Opisthorchis* antigens from intermediate snail-extract (Sephacryl S-200) (Waikagul *et al*, 2002). Charge fractionation of antigens by isoelectric focusing has been used for *Taenia solium* metacestodes (Ko and Ng, 1998; Ito *et al*, 1998) and echinococcus antigens (Ito *et al*, 1999). In this study, we purified antigens from male and female adult *D. immitis* worms by two simple methods: gel filtration chromatography for size fractionation of antigens and iso-electrofocusing (IEF) for charge fractionation. The practical applicability of using those antigen fractions was analyzed by IgG-ELISA in bancroftian filariasis patients' sera, in patients with various parasitic diseases and in normal healthy control sera. These results were compared to the results of detecting circulating filarial antigen using test kit, Og4C3-ELISA.

MATERIALS AND METHODS

Serum samples

Serum samples used in this study were selected from previous samples obtained over the past few years at the Immunodiagnostic Unit, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University. All serum samples have been kept at -35°C to -70°C. Thirty samples of *W. bancrofti* proven by blood film tests were used. To test cross-reactivity, 131 cases with other parasitic infections included parasitologically confirmed cases of nematodiasis ($N = 47$), cestodiasis ($N = 16$), trematodiasis ($N = 24$) and protozoan infections ($N = 4$) were examined. Other cases diagnosed by parasitological, immunoblot

examinations, clinical findings or historical data were trichinellosis ($N = 10$), angiostrongyliasis ($N = 10$), toxocariasis ($N = 6$), neurocysticercosis ($N = 10$), and cystic echinococcosis ($N = 4$). The sera of 30 healthy controls who tested negative for all parasites by fecal examination were included. All healthy controls were collected from areas non-endemic for filariasis used in previous research (Dekumyoy *et al*, 2000a).

Parasite collection

The dog hearts were obtained in San-Pa Tong District, Chiang Mai Province, where dog meat is a favored food. Adult *D. immitis* worms were collected from the right ventricles and pulmonary arteries of the hearts. The worms were washed with normal saline solution many times on a rotating shaker, then with distilled water, and kept at -70°C until used.

Crude antigen

The crude somatic antigens were prepared separately from both sexes of frozen adult *D. immitis* worms, using the extraction procedure of Dekumyoy *et al* (2000b) with modification. The frozen worms were allowed to thaw to room temperature before extraction. Then, the suspension was sonicated with a Super sonicator (Ultrasonic Processor XL) fitted with probe No. 419B, at magnification No. 4, at 1 minute intervals for 15 minutes, then centrifuged at $20,000g$ for 45 minutes at 4°C . The supernatant was dialyzed and concentrated by an Amicon PM 10 membrane. The protein content of the two antigens was determined by a Coomassie Plus Protein Assay Reagent Kit (Pierce).

Gel filtration chromatography

Five milliliters (25 mg) of crude somatic male and female antigens were fractionated by pre-swollen Sephacryl S-200 (Pharmacia LKB, Uppsala, Sweden) by

column chromatography (Pharmacia LKB). The separation followed the process of Dekumyoy *et al* (2004). The optical density of each fraction was graphically plotted to determine peak patterns. All fractions in each peak were pooled, dialyzed, and concentrated with the Amicon PM10. The protein content of the each peak was determined. All peaks were determined for selecting a proper antigen by checkerboard titration.

Isoelectric focusing (IEF)

Crude somatic male and female antigens were separately fractionated by IEF using a Rotofor cell (Bio-Rad), according to Ito *et al* (1997) and the Bio-Rad protocol. Ten milliliters of crude male (50 mg) and female (100 mg) antigens were mixed in the ampholyte solution, pH 3-11. The isoelectric points of the extracts were generated using 12 watts at 4°C . After 4-5 hours, all 20 fractions were aspirated by a vacuum pump into serial collection tubes. Individual fractions were determined for pH, dialyzed against distilled water, and their protein contents were determined.

After isoelectric focusing male and female antigens were separately fractionated into 20 fractions each and the immunoreactivity of each fraction was tested by the methods of Voller *et al* (1979) by comparing ELISA-OD ratios between pooled bancroftian filariasis samples and healthy control sera. In brief, each fraction was diluted to 2 mg/ml and reacted with positive or negative sera (1:200), washed, and then incubated with dilute anti-human IgG at 1:2,000. Although Voller *et al* (1979) recommended selecting an antigenic fraction having an OD ratio $> 5:1$, the highest OD ratio of the charge-fractionated male antigen was 3.4 for Iso-MF10 and 3.3 for Iso-FmF14 with the female antigenic fractions. Those two fractions, one each for male and female antigens, were selected

for the ELISA.

Indirect ELISA

Indirect-ELISA was performed in a microtiter plate (Nunc, Denmark), as described by Dekumyoy *et al* (1998) with minor modification. The reactions were visualized with substrate ABTS substrate [2, 2-azino-di-(3-ethyl-benzthiazoline sulfonate, Sigma)] after 30 minutes incubation. Absorbance was measured at 405 nm after 1% SDS was added to stop the reaction.

Capture-ELISA

The 96 well microtiter plates were prior coated with monoclonal antibody (Og4C3, TropBio, Australia) for detecting *W. bancrofti* antigen. The test and interpretation were carried out according to the manufacturer's instructions.

RESULTS

Immunoreactivity of crude antigen

The immunoreactivity of crude somatic antigens of male and female *D. immitis* adult worms was examined against sera from patients with bancroftian filariasis, other parasitic infections and healthy controls at the optimal conditions for the ELISA. Using a mean OD of 0.178 for the healthy control sera as the cut-off value, sensitivity, specificity, positive and negative predictive values for crude male antigen were 100, 60.8, 32.3, and 100%, respectively. The cross-reactivity of the crude male antigen with sera from patients with other parasite infections was 37.4% (49/131). When cross-reactivity with healthy controls (5/30) was included, the total cross-reactivity was 39.1% (63/161). Although cross-reactivity with other parasitic infections was observed in 49 involving 13 diseases, all sera from patients with sparganosis, hymenolepiasis nana, paragoni-

miasis heterotremus, haplorchiasis, giardiasis, and amebiasis gave OD values below the cut-off level.

With crude female antigen, the sensitivity, specificity, positive and negative predictive values were 80, 52.8, 24, and 93.4%, respectively, with a cut-off value of 0.305 (mean OD of healthy control). Cross-reactivity with all serum samples was 47.2% (76/161) involving 15 diseases, including the data for healthy controls. Similar to the results for the crude male antigen, all sera from patients with sparganosis and paragonimiasis heterotremus gave OD values below the cut-off level. Baseline data for both crude antigens against all serum samples are presented in Table 1.

Gel chromatography antigen fractions

After Sephacryl S-200 gel chromatography, the crude male antigen was separated into 3 fractions according to the elution profile of the protein concentration peaks (Fig 1a), the fractions were named MP1, MP2, and MP3. The crude female antigen gave 5 peaks, the fractions were named FmP1, FmP2, FmP3, FmP4, and FmP5 (Fig 1b).

Immunoreactivity of size-fractionated antigens

Of the male fractions MP1-MP3, MP1 showed the best discrimination between positive bancroftian sera and healthy control sera on checkerboard titration. Of the female antigens FmP1-FmP5, FmP1 gave the best resolution between positive and negative sera. Therefore, MP1 and FmP1 were selected for further analyses. The conditions for the ELISA with MP1 were as follows: 4 µg/ml of antigen, a 1:400 dilution of serum and a 1:2,000 dilution of anti-human IgG. The ELISA conditions used for FmP1, were the same as those for MP1 except for a 1:1,000 dilution of anti-human IgG.

Table 1
 Demonstration of cross-reactivity among serum antibodies of heterologous diseases and antigenic products from male and female crude antigens, partially purified antigens, MP1 and FmP1, and Iso-electric focusing antigens, Iso-MF10, and Iso-FmF14, following their cut-off values.

Diseases (No.)	Crude male (0.178)		Crude female (0.305)		MP1Ag (0.582)		FmP1Ag (0.411)		Iso-MF10 (0.347)		Iso-FmF14 (0.253)	
	FP	%FP	FP	%FP	FP	%FP	FP	%FP	FP	%FP	FP	%FP
Gnathostomiasis (10)	4	40	4	40	-	-	3	30	-	-	1	10
Strongyloidiasis (10)	10	100	9	90	1	10	7	70	-	-	8	80
Hookworm infections (10)	2	20	4	40	-	-	1	10	-	-	-	-
Trichinellosis (10)	9	90	10	100	1	10	8	80	1	10	6	60
Capillariasis (3)	3	100	3	100	-	-	3	100	1	33.3	3	100
Angiostrongyliasis (10)	6	60	6	60	3	30	6	60	1	10	4	40
Ascariasis (7)	3	42.8	4	57.1	-	-	1	14.3	-	-	1	14.3
Trichuriasis (7)	1	14.3	3	42.8	-	-	-	-	-	-	1	14.3
Toxocariasis (6)	2	33.3	2	33.3	1	16.7	3	50	-	-	1	16.7
Neurocysticercosis (10)	2	20	6	60	-	-	4	40	-	-	5	50
Cystic echinococcosis (4)	4	100	4	100	1	25	3	75	1	25	4	100
Taeniasis (10)	2	20	5	50	-	-	1	10	-	-	1	10
Sparganosis (3)	-	-	1	33.3	-	-	1	33.3	-	-	3	100
Hymenolepiasis nana (3)	-	-	-	-	-	-	-	-	-	-	1	33.3
Paragonimiasis heterotremus (9)	-	-	1	11.1	-	-	5	55.5	-	-	6	66.7
Opisthorchiasis (10)	1	10	1	10	1	10	2	20	1	10	3	30
Haplorchiasis taichui (5)	-	-	-	-	-	-	4	80	-	-	4	80
Giardiasis (2)	-	-	-	-	-	-	-	-	-	-	1	50
Amoebiasis (2)	-	-	-	-	-	-	-	-	-	-	-	-
Total number (131)	49	37.4	63	48.1	8	6.1	52	39.7	5	3.8	53	40.4
Healthy controls (30)	14	46.7	13	43.3	-	-	13	43.3	-	-	5	16.7
Total number (161)	63	39.1	76	47.2	8	4.9	65	40.4	5	3.1	58	36

Table 2

Comparison of evaluation of IgG-ELISA (antibody detection) and Og4C3 (circulating filarial antigen detection), including false positive and false negative from the tests.

Detection	IgG-ELISA		Og4C3-ELISA
	<i>D. immitis</i> worms		Circulating Ag of <i>W. bancrofti</i>
	Ab/MP1	Ab/Iso-MF10	
Sensitivity	100%	100%	86.7%
Specificity	95%	96.9%	99.4%
Positive predictive value	78.9%	85.7%	96.3%
Negative predictive value	100%	100%	97.6%
False negative	none	none	4
False positive	trichinellosis (1) toxocariasis (1) angiostrongyliasis (3) cystic echinococcosis (1) opisthorchiasis (1) strongyloidiasis (1)	trichinellosis (1) capillariasis (1) angiostrongyliasis (1) cystic echinococcosis (1) opisthorchiasis (1)	neurocysticercosis (1)

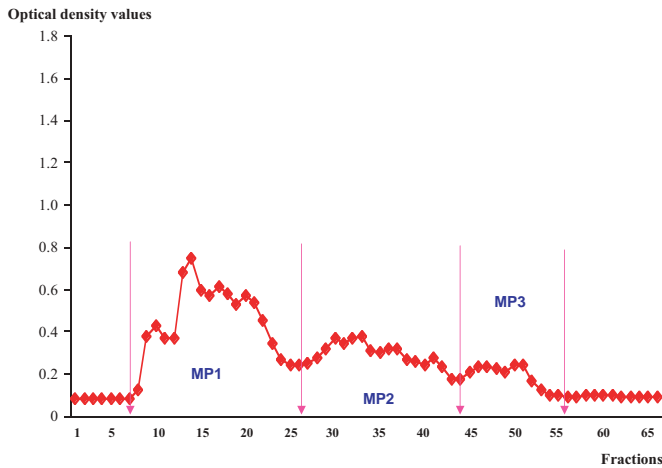
After the ELISA was carried out against all serum samples, MP1 yielded 100% sensitivity, 95% specificity, 78.9% positive predictive value, and 100% negative predictive value, at a cut-off value of 0.582 (mean OD of healthy controls + 3SD). Cross reactivity with other serum samples with other parasitic diseases was 6.1% (8/131) comprising 6 diseases: strongyloidiasis, angiostrongyliasis, toxocariasis, trichinellosis, cystic echinococcosis, and opisthorchiasis, and 4.9% (8/161) with healthy controls (0/30) (Fig 3, Tables 1, 2).

FmP1 gave 100% sensitivity, 59.6% specificity, 31.6% positive and 100% negative predictive values at a cut-off value of 0.411 (mean + 3SD of the healthy control). Cross-reactivity with serum samples of other parasitic diseases was 40.4% (65/161) comprised of 15 diseases: gnathostomiasis, strongyloidiasis, hookworm infections, trichinellosis, capillariasis, angiostrongyliasis, ascariasis, toxocariasis, taeniasis,

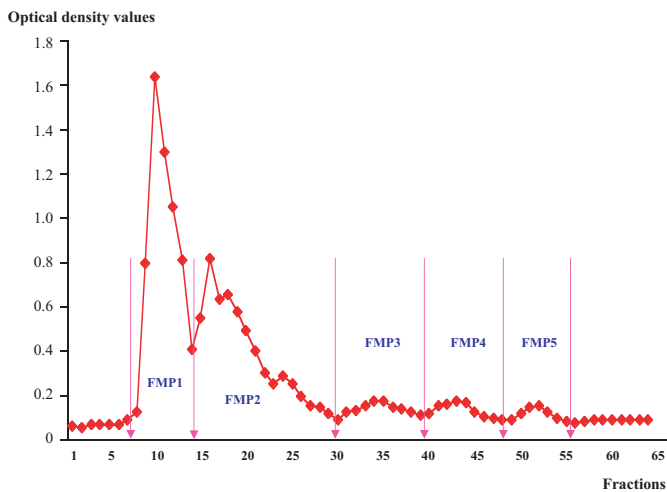
sparganosis, cystic echinococcosis, neurocysticercosis, paragonimiasis heterotremus, opisthorchiasis, and haplorchiasis taichui (Table 1).

Isoelectric focusing fractionation

After isoelectric focusing (charge) separation of the male and female crude antigens on a Rotofor cell (Bio-Rad), the male antigens dispersed at a pH range of 3.5-8.9 and the female antigens at a pH range of 3.4-9. After charge separation, the materials in the cell were mechanically fractionated into twenty fractions for each sex, named Iso-MF1 to Iso-MF20 for male antigen fractions and Iso-FmF1 to Iso-FmF20 for female antigen fractions. Similar to preliminary screening, the immunoreactivity of each fraction was determined as the OD ratio of ELISA against pooled filariasis-positive and -negative sera. Consequently, Iso-MF10 (pH 7.5) and Iso-FmF14 (pH 7.6), showing the highest OD ratios of 3.4 and 3.3, respectively,



a



b

Fig 1-Partial purification profile of *D. immitis* antigens, crude male (a) and crude female (b) obtained from Sephacryl S-200 gel chromatography. The crude male profile was separated into three peaks and the female profile into five peaks.

(Fig 2) were selected for further analyses.

Immunoreactivity of charge-fractionated antigens

The immunoreactivity of the Iso-MF10 fraction was tested against serum samples from patients with bancroftian filariasis, other parasite infections, and healthy controls. The sensitivity, specificity, positive

and negative predictive values for the test were 100, 96.9, 85.7, and 100%, respectively, at a cut-off value of 0.347 (mean + 3SD of normal healthy controls). Cross-reactivity was 3.8% (5/131), with false-positive results for 5 helminthiasis sera: trichinellosis, capillariasis, angiostrongyliasis, cystic echinococcosis and opisthorchiasis (Fig 4, Tables 1, 2). The same panel of the sera, Iso-FmF14 gave 100% sensitivity, 64% specificity, 34.1% positive predictive value, and 100% negative predictive value, at a cut-off value of 0.253 (mean + 3SD of normal healthy controls) by ELISA. Cross-reactivity occurred with 17 diseases at a rate of 40.4% (53/131), this became 36% (58/161) when the data of healthy controls (5/30) were included (Table 1).

Capture-ELISA

The test kit detected *W. bancrofti* antigen with sensitivity, specificity, positive and negative predictive values of 86.7, 99.4, 96.3 and 97.6%, respectively. A false positive was found with neurocysticercosis following allocation of sample to the titer groups (TropBio protocol). Most filariasis sera had high levels of *W. bancrofti* antigen (Table 2).

DISCUSSION

Crude antigens gave unsatisfactory specificity, especially when an array of sera from patients with different parasitic diseases was used. In the present study, crude *Dirofilaria immitis* male and female antigens gave specificities of 60.8% and 52.8%,

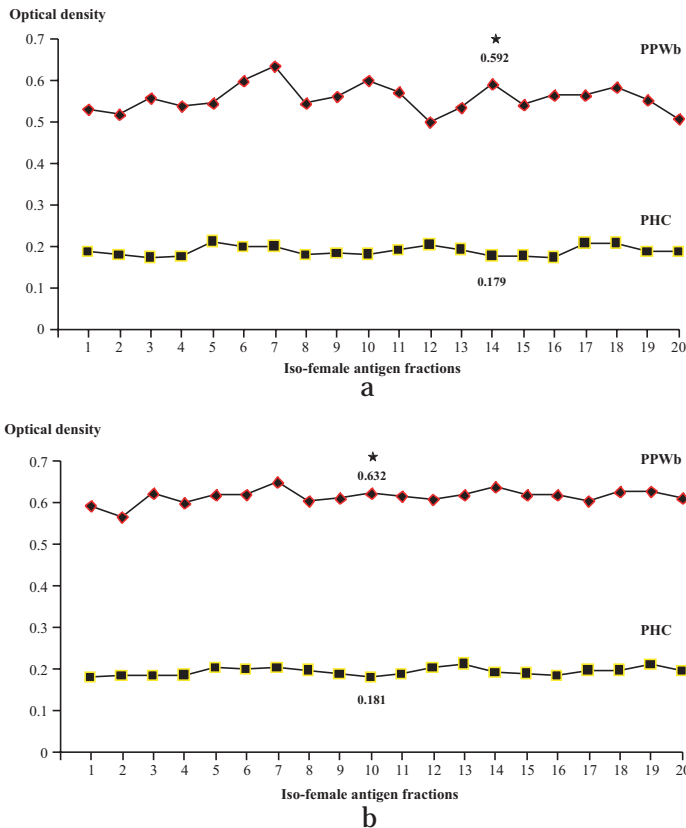


Fig 2—Graphic comparison of OD-ELISA values between pooled positive sera of bancroftian filariasis and healthy controls by reaction with 20 fractions of iso-electricfocusing female (a) and male (b) antigens, of which, fraction 14 of the female antigen and fraction 10 of the male antigen show the highest OD ratio values.

respectively, sensitivities of 100% and 80%, respectively. Harnnoi *et al* (1996) reported high cross-reactivity between adult female *D. immitis* crude antigens and antibodies from gnathostomiasis, opisthorchiasis, paragonimiasis, capillariasis and other intestinal helminthiases, including trichinellosis (78.3%), and angiostrongyliasis (85%) with IgG-ELISA. In our study, trichinellosis and angiostrongyliasis sera frequently cross-reacted with crude *D. immitis* adult male and female antigens. The frequency of cross-reactivity was far higher with female *D. immitis*

crude antigen than with *D. immitis* male antigen. The reason for this higher cross-reactivity may be because the female antigen was carrying microfilarial antigen. *D. immitis* microfilaria antigens cross-react with bancroftian filariasis using counterimmunoelectrophoresis (Desowitz and Una, 1976) and immunoelectrophoresis (Wheeling and Hutchison, 1971).

Molecular sieve gel-chromatography has been used for the separation of some parasite antigens to determine antigens with better specificity for immuno-diagnosis. Sero-diagnosis using fractionated antigens may give variable various sensitivities and specificities which are not completely satisfactory. In the present study, the sensitivities of crude male and female *D. immitis* adult worm antigen were 100% and 80%, and their specificities were 60.8% and 52.8%, respectively. After Sephacryl S-200 gel filtration, the male antigen fraction MP1 and the female antigen fraction FmP1 gave the specificities of

95% and 60%, respectively, with both sensitivities of 100%. Gel filtration chromatography is a simple, rough separation method based on molecular size, the relatively high specificity of MP1Ag with the high sensitivity makes it a good candidate as a diagnostic antigen.

Compared with molecular sieve chromatography, liquid phase iso-electric-focusing (IEF) has been used for bio-separation to produce greater levels of purified antigen. IEF yielded better antigen fractions for the diagnosis of neurocysticercosis (Ito *et al*, 1998), and cystic and alveo-

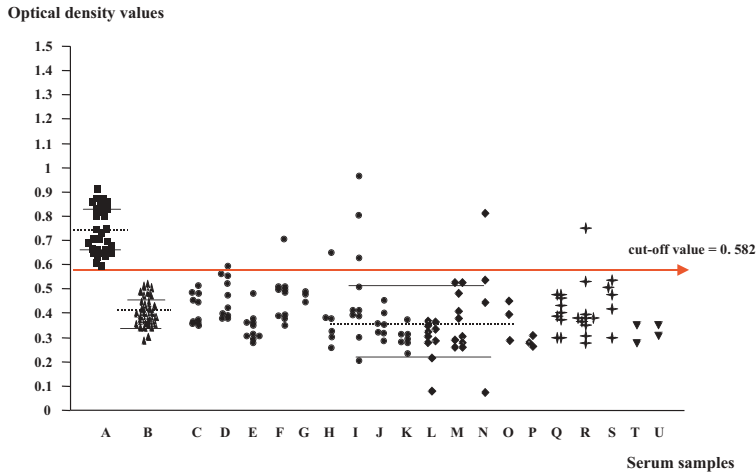


Fig 3–Scatter plot patterns of ELISA optical densities using MP1Ag against serum samples: A, bancroftian filariasis; B, healthy serum controls; C, gnathostomiasis; D, strongyloidiasis; E, hookworm infection; F, trichinellosis; G, capillariasis; H, toxocariasis; I, angiostrongyliasis; J, ascariasis; K, trichuriasis; L, taeniasis; M, neurocysticercosis; N, cystic echinococcosis; O, sparganosis; P, hymenolepiasis nana; Q, paragonimiasis heterotremus; R, opisthorchiasis; S, haplorchiasis taichui; T, giardiasis; U, amebiasis. Bars on three groups: bancroftian filariasis, healthy controls and other parasitic diseases; (—), low and high values at mean plus SD; (----), mean values.

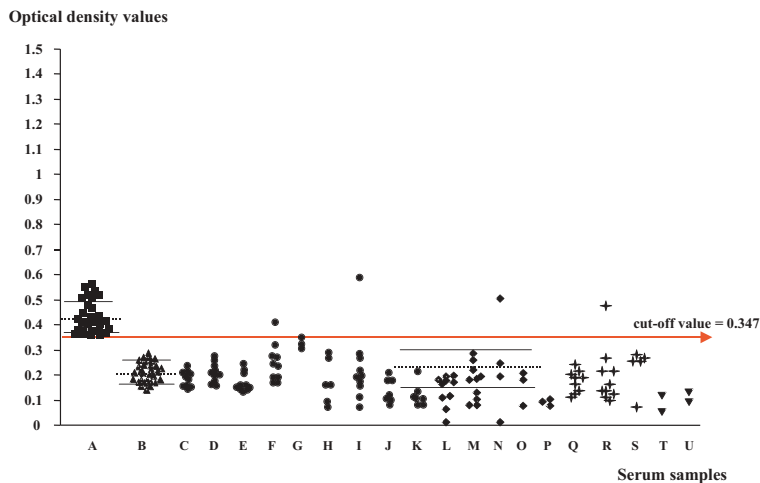


Fig 4–Scatter plot patterns of ELISA optical densities using Iso-MF10 against serum samples: A, bancroftian filariasis; B, healthy serum controls; C, gnathostomiasis; D, strongyloidiasis; E, hookworm infection; F, trichinellosis; G, capillariasis; H, toxocariasis; I, angiostrongyliasis; J, ascariasis; K, trichuriasis; L, taeniasis; M, neurocysticercosis; N, cystic echinococcosis; O, sparganosis; P, hymenolepiasis nana; Q, paragonimiasis heterotremus; R, opisthorchiasis; S, haplorchiasis taichui; T, giardiasis; U, amebiasis. Bars on three groups: bancroftian filariasis; healthy controls and other parasitic diseases; (—), low and high values at mean plus SD; (----), mean values.

lar echinococcosis (Ito *et al*, 1999), etc. In the present study, the male antigen fraction Iso-MF10, prepared by IEF with Rotofor cell separation, yielded very high specificity (96.9%). Cross-reactivity was observed in only 5 cases: trichinellosis, angiostrongyliasis, capillariasis, cystic echinococcosis, and opisthorchiasis. These cases could reflect either cross-reactivity or co-infection with *D. immitis*. Although human dirofilariasis in Thailand is rarely reported but *D. immitis* is found in dogs in Thailand (Niwetpathomwat *et al*, 2007; Boonyapakorn *et al*, 2008). Iso-MF10 may still be used as diagnostic antigen for bancroftian filariasis, because the cross-reactive parasitic diseases can easily be distinguished from filariasis by their clinical symptoms.

This study also compared the results from antigens, MP1 and Iso-MF10, with that of Og4C3-ELISA. The Og4C3-ELISA and immunochromatographic test (ICT), have been used for detection of circulating antigens of *Wuchereria bancrofti*. Varying sensitivities and specificities have been seen with these kits. The Og4C3-ELISA test has been found to be more accurate in the diagnosis of bancroftian filariasis than finding microfilaria in blood samples (More and Copeman, 1990; Chanteau *et al*, 1994; Itoh *et al*, 1999; Nuchprayoon *et al*, 2001; 2003; Shah and Mulla, 2007). In contrast, the ICT has been found to be more sensitive than Og4C3 (Pani *et al*, 2004) and less sensitive than Og4C3-ELISA (Nuchprayoon *et al*, 2001, 2003). Both the Og4C3 and ICT tests yielded similarly high sensitivities (96.69%) for microfilarial serum samples (Rocha *et al*, 2009). Non-commercial sero-tests have also been used to detect filarial antibodies, some with good results. An IgG4 urine ELISA used *Brugia pahangi* as an antigen for detection. The test had a high sensitivity (95.5%) for

antigen- and/or microfilaria-positive status, and a specificity of 99% for non-endemic controls in Thailand, Lao PDR, and Japan. The Og4C3 urine ELISA gave 95.6% positivity for cases of bancroftian filariasis (Itoh *et al*, 2001). The reason for the differing results between the ICT and urine ELISA in Thailand is unknown; antigenemia among post-treatment samples dropped drastically from 100% by ICT card strip (pre-treatment) to 32.8% by Og4C3 (post-treatment). When these pre- and post-treatment samples were analyzed using urine ELISA, the pre-treatment 37.3% dropped slightly to 35.8% (Kimura *et al*, 2003). Both test kits appear to be very sensitive and specific for bancroftian filariasis, with similar results (Weil *et al*, 1997; Simonsen and Dunyo, 1999), and for noting antigenemia-positive cases reported years post-treatment (Zheng *et al*, 1990; Eberhard *et al*, 1997). Therefore, the quality of the urine ELISA and the Og4C3 ELISA should be good enough to produce similar results post-treatment. The test kits to detect circulating *W. bancrofti* antigens yield controversial specificity and sensitivity detection results (Rocha *et al*, 1996, 2009). In our study, 4 microfilaria cases were false negative with Og4C3-ELISA while the same serum samples were positive by Iso-MF10 antigen. The occurrence of 4 false negatives may be related to low CFA levels and low numbers of microfilaria in the serum samples in our study. The Iso-MF10 antigen has several antigenic molecules useful for detecting anti-filarial antibodies based on IgG-ELISA testing.

In conclusion, the MP1 antigen, partially purified by gel chromatography, and the Iso-MF10, fractionated by iso-electrofocusing, are candidate antigens which yield high sensitivity and specificity levels, and should prove useful as clinical

diagnostic aids for detecting bancroftian filariasis. Cross-reactivity occurs in some cases with other disease. It would be interesting to continue analysis of these antigens with the IgG4-ELISA in the future.

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