FIELD TRIAL OF THE ICT FILARIASIS FOR DIAGNOSIS OF *WUCHERERIA BANCROFTI* INFECTIONS IN AN ENDEMIC POPULATION OF THAILAND

Adisak Bhumiratana[1], Surachart Koyadun[1,2], Saravudh Suvannadabba[3], Kobkarn Karnjanopas[3], Jirasak Rojanapremsuk[1], Prayute Buddhirakkul[1] and Worawit Tantiwattanasup[4]

[1]Department of Parasitology, Faculty of Public Health, Mahidol University, Bangkok, Thailand; [2]Regional of Vector Borne Disease Control 4, Songkhla, Thailand; [3]Filariasis Division, Department of Communicable Disease Control, Ministry of Public Health, Nonthaburi, Thailand; [4]Umphang Hospital, Ministry of Public Health, Tak, Thailand

Abstract. The ICT Filariasis, a rapid card test format, which is based on qualitative detection by monoclonal antibody of the circulating antigen of *Wuchereria bancrofti* adult worm, is a new diagnostic test of choice for determining the infections under field conditions. By using clinical and recall techniques and microscopy (thick smear and capillary tube technique) as reference, we assessed the efficiency of the ICT card test in sera from 225 subjects living in *W. bancrofti*-endemic villages of Tak Province, Thailand, who were recruited during a cross-sectional community survey. The ICT card test gave a 20% antigen positive rate, while other tests gave lower positive rates of the same 5.8% by clinical and recall techniques and thick smear, and 5.3% by capillary tube technique, respectively. The ICT card test had a specificity of 100% when sera from microfilaremic subjects were positive, as when sera from *W. bancrofti* non-endemic subjects either with *Brugia malayi* microfilaremia or with other parasites, and those from normal controls were all negative by the test. When done in *W. bancrofti* microfilaremia sera, the ICT card test had a sensitivity of 100% using a microscopy as reference, and 84.6% when using clinical and recall techniques. However, the ICT card test was more sensitive than the others when done in endemic normal sera (14% positive). Such findings compared well with findings in endemic area of South America, suggested its usefulness to detect *W. bancrofti* infections in endemic area, especially on the Thai-Myanmar border.

INTRODUCTION

Lymphatic filariasis is thought to infect at least 120 million people in more than 70 endemic countries (WHO, 1996). *W. bancrofti* is a major cause of lymphatic filariasis burden leading to a broad spectrum of clinical mainfestations to permanent disability. Since most endemic countries do not use or develop effective epidemiologic tools as primary surveillance for bancroftian filariasis. Meanwhile more informative filariasis burden has been needed for national action plan and execution of lymphatic filaria control activity. These could reflect accurate estimates of national, regional and global burden of bancroftian or brugian filariasis as well (Ottesen *et al*, 1995). Depending on the traditional method such as thick smear which is simple, low technology and cost, and still the best method in the field work (WHO, 1992). However, like microfilaria detection by membrane filtration or by PCR assay, diagnosis of *W. bancrofti* infection under field conditions is less effective due to practical and biological limits and oftenly less promising for monitoring the effectiveness of diethylcarbamazine (DEC) treatment (William *et al*, 1996). An alternative immunodiagnostic test such as the immunochromatographic test (ICT) for detection of the circulating antigen of *W. bancrofti* adult worm has been proven useful in the rapid card test format, relatively similar to other antigen tests (Weil *et al*, 1997), which works perfectly with sera from most asymptomatic microfilaremic patients from different endemic areas. Recently, the ICT card test has provided rapid assessment for determining prevalence of *W. bancrofti* infections under field conditions in South America (Freedman *et al*, 1997). We describe here the results of more extensive foci on evaluating the performance of the ICT FILARIASIS done in sera from an endemic population of Thailand where malaria and bancroftian filariasis coexist and no study had been conducted. The performance of the ICT FILARIASIS was assessed using clinical and recall techniques (Ottensen *et al*, 1995) and microscopic examination (thick smear and capillary tube technique) (WHO,1992; Phantana *et al*, 1997) which

Correspondence: Adisak Bhumiratana, Department of Parasitology, Faculty of Public Health, Mahidol University, Bangkok 10400, Thailand.
Tel/ Fax: (66-2)-644-5130

both are common techniques for night blood survey in the area (Suvannadabba, 1993) as reference.

MATERIALS AND METHODS

Selection of subjects

A cross-sectional community survey (Bhumiratana *et al*, manuscript in preparation) was carried out in association with teams of Filariasis Division and Center of Vector Borne Disease Control 18 (Mae Sot) of the Department of Communicable Disease Control, Ministry of Public Health, during March-May 1998. The subjects resided in *W. bancrofti*-endemic villages of Mae Chan subdistrict, Umphang district, Tak Province on the Thai-Myanmar border. They were informed about the purpose of this study and consented to participate. The 225 subjects recruited into the study were interviewed and physically examined. Night blood samples were then collected by venepuncture between 18.00-24.00 hours as high preferential time of the nocturnal subperiodic microfilariae (Khamboonrung *et al*, 1987; Sitthai, 1988) for parasitological and immunological assessments. Single stool samples were collected, preserved in 10% formalin and transferred to laboratory for examination of intestinal helminth infections by formalin-ether sedimentation technique (Zaman *et al*, 1994). Following physical and microscopic examinations, the subjects were categorized into four groups: 1) asymptomatic microfilaremic subjects, 2) symptomatic microfilaremic subjects, 3) symptomatic amicro-filaremic subjects, 4) asymptomatic amicrofilaremic subjects (or 'endemic normals').

Clinical and recall techniques

The modified uniform physical examination and recall questioning (WHO, 1987) were composed of three main lines as follows: 1) Baseline demographic characteristics of subjects such as sex, age, residence time, previous history of bancroftian filariasis and of DEC treatment were collected by interview. The subjects who had past history of thick smear positive and were given any medical managements (DEC treatment, surgical management, etc) were considered to have previous history of bancroftian filariasis. 2) The subjects who had past history of febrile episode like lymphadenitis with retrograde lymphangitis and fever for days in the past three months or longer, were considered to have active infection. Enlarged lymph nodes (over 1.5 cm in diameter of inguinal or axillary lymph nodes) with any signs and symptoms of pain, tenderness, redness, local warmth and local swelling, were considered as acute stage of infection. 3) The subjects with chronic lymphatic pathology like female subjects with vulva elephantiasis or male subjects with chronic hydrocele (grade 1-3) with any signs of chyluria or hematuria were considered to have chronic obstructives in the past 3-5 years. Those subjects with leg elephantiasis (grade 1-3) were also considered to have culminating lymphedema in the past 3-5 years.

Following evaluation, subjects were categorized into two groups: 1) symptomatic subjects who had any clinical manifestations of acute or chronic lymphatic pathology, 2) asymptomatic subjects who had no clinical presentations cited above.

Capillary tube technique

Briefly, ~ 60-µl venous blood sample was drawn into heparinized capillary tube, sealed with wax and then centrifuged at 1,200 rpm for 5 minutes. The motile microfilariae were visualized in plasma or corpuscle fraction under microscopic fields with 40x magnification. Hematocrit capillary tube was declared as a negative tube after viewing entirely whole fractions.

Thick smear

Briefly, 60-µl venous blood adjusted by pipetting was smeared on to duplicate microscope slides and an ellipsoidal smear (a dimension of 5.0 x 1.5 cm) was made, air-dried and then stained with Giemsa's solution. In a positive slide, species identification and microfilarial counts were performed in the laboratory. The Giemsa-stained microfilariae of *W. bancrofti* were visualized under microscopic fields with 100x magnification. Duplicate blood films were declared as negative slides after viewing entirely whole blood smears.

ICT Filariasis

The test was performed according to the manufacturer's instruction. The ICT Filariasis Test Kit (ICT Dignostics, Balgowlah, NSW, Australia) used two antibodies; one was an anti *W. bancrofti* antigen polyclonal antibody (PAb) gold conjugate and second antibody was a monoclonal antibody (MAb) specific for *W. bancrofti* antigen. The 50-µl serum sample prepared from clotting blood from endemic individuals (either from non-endemic individuals) was added on to the pink pad of the card test to allow any *W. bancrofti* antigen present to bind to PAb gold conjugate. Two drops of reagent A (supplied with the test kit) were added to the white pad. When the card test was closed, the sample and the PAb gold conjugate on the pink pad were

Table 1
Comparison of the four test results by baseline demographic characteristics of the subjects.

Characteristics	Sample no.	Samples positive (%)			
		ICT card test	Thick smear	CAP	CRT
Total	225	45 (20.0)*	13 (5.8)*	12 (5.3)*	13 (5.8)*
Sex					
Male	135	31	9	8	11
Female	90	14	4	4	2
Age (years)					
< 15	3	1	0	0	2
15-29	119	15	1	2	1
30-44	76	20	8	8	6
45-59	22	8	3	3	3
≥ 60	5	1	0	0	1
X ± SD	29 ± 12.9	32.9 ± 12.1	35.7 ± 11.0	37.5 ± 9.5	35.4 ± 18.2
(min-max)	(4-69)	(4-60)	(15-55)	(18-55)	(4-69)
Residence time (years)					
< 5	16	4	2	2	3
5-24	115	18	4	4	3
25-44	77	16	5	4	4
45-64	17	7	2	2	3
X ± SD	23.5 ± 12.8	25.8 ± 15.4	24.9 ± 16.6	24.0 ± 17.0	26.1 ± 19.5
(min-max)	(0.08-60)	(0.08-60)	(1-55)	(1-55)	(1-55)
Previous history of bancroftian filariasis					
Yes	13	6	3	3	3
No	212	39	10	9	10
Previous history of DEC treatment					
Yes	12	5	2	2	3
No	213	40	11	10	10

CAP = capillary tube technique; CRT = clinical and recall techniques
* statistically significance with p-value < 0.001 (Cochran Q = 73, df = 3)

transferred to the end of the membrane and then migrated up crossing the immobilized MAb line (referred to as Test). The result was read through the viewing window of the card test within 45 minutes. In a positive serum sample, any *W. bancrofti* antigen complexed with PAb gold conjugate was captured by the MAb on the membrane, and two pink lines formed: Test and Control were visible. Pale pink color on the MAb line was considered as positive. In a negative serum sample, no PAb gold conjugate was captured by the MAb on the membrane, and a pink line formed: only Control was visible.

All tests were done independently by examiners and the test results were all blind.

Data analysis

The four test results of determining infection rates were compared using Cochran Q test (Zar, 1984). Sensitivity was analysed using microscopy or clinical and recall techniques as reference. Specificity was analysed using sera from *W. bancrofti* non-endemic subjects with *B. malayi* and with other intestinal helminth infections, and those from normal controls.

RESULTS

All tests (thick smear, capillary tube technique, clinical and recall techniques and ICT card test) were assessed in 225 subjects. Of the 225 which were assessed by microscopic examination, 13 (5.8%) were parasitologically found by thick smear, 12 (5.3%) were also found by capillary tube technique (Table 1). In one of these microfilaremia positive cases, it

Table 2
Performance of the ICT card test with serum.

Parasitological group	Sample no.	Samples positive by ICT card test (% positive)
***W. bancrofti* endemic**		
Mf +ve [a]	14	14 (100)
***W. bancrofti* non-endemic** [b]		
Brugia malayi Mf +ve	10	0 (0)
Opisthorchis viverrini	5	0 (0)
Hookworms	10	0 (0)
Normal controls [c]	20	0 (0)

[a] All were sera from 14 microfilaremic (Mf) subjects including 8 with hookworms, 1 with *Ascaris lumbricoides*, 2 with mixed infections, 2 without intestinal helminth infections and 1 (whose stool sample was not obtained). [b] All sera were obtained from the subjects including [c] 20 normal persons negative by stool examination, who had no experience of bancroftian filariasis.

Table 3
Comparison of the ICT card test in serum with the results of microscopy and clinical and recall techniques.

Clinical group	Sample no. (N = 225)	Samples positive by ICT card test (% positive)
Symptomatic microfilaremic subjects	7	7 (100)
Asymptomatic microfilaremic subjects	7	7 (100)
Symptomatic amicrofilaremic subjects	6	3 (50.0)
Endemic normals	205	28 (13.7)

was negative by thick smear but positive by capillary tube technique and, therefore, 14 in all were positive by microscopy. Of the 225, 13 (5.8%) were clinically found by clinical and recall technique, 45 (20.0%) were found antigenemic by ICT card test in serum. Among 45 antigenemia positive, the same 7 were found microfilaremic symptomatic and asymptomatic, 3 were found symptomatic amicrofilaremic and 28 were found asymptomatic amicrofilaremic, respectively (Table 3). The four test results were statistically significant difference with p-value <0.001.

The ICT card test had a specificity of 100%. Fourteen sera from *W. bancrofti* microfilaremic subjects with and without intestinal helminth infections were positive whereas sera from *W. bancrofti* non-endemic subjects including 10 with *B. malayi* microfilaremia, 5 with *O. viverrini*, 10 with hookworms and 20 normal control sera were all negative by the ICT card test (Table 2). When done in sera from microfilaremic subjects with and without clinical filariasis, the ICT card test had a sensitivity of 100% using microscopy as reference (Table 3). When done in clinical disease sera, the ICT card test had a sensitivity of 84.6% using clinical and recall techniques as reference (Table 4). However, the ICT card test had more sensitivity than other tests when done in endemic normal sera (14% positive) (Table 3).

DISCUSSION

The rapid qualitative assessment by the ICT card test is dependent on a monoclonal antibody AD12.1 specific for *W. bancrofti* circulating antigen present in human serum not required for pretreatment (Weil *et al*, 1997). Hence, we used the ICT card test for CFA detection in an endemic population. In most positive serum samples, pink color developed on the MAb line after 5 minutes but better results could take longer time (Weil *et al*, 1997). Because of large number of endemic normals (91% of subjects) who were asymptomatic amicrofilaremic, clear-cut antigen-negative test result was therefore declared within 45 minutes in order to increase accurate assessment of the sensitivity and the speci-

Table 4
Comparison of the ICT card test in serum with the results of clinical and recall techniques.

Clinical group	Sample no. (N = 225)	Samples positive by ICT card test (% positive)
Asymptomatic filariasis	212	34 (16.0)
Symptomatic filariasis	13	11 (84.6)
Enlarged inguinal lymph nodes	2	2
Adenolymphangitis	1	1
Hydrocele	6	3
Hydrocele and enlarged inguinal lymph nodes	5	5

ficity.

The specificity of the ICT card test in previous study was greater than 99% (Weil *et al*, 1997). In present study it had a specificity of 100% when only sera from *W. bancrofti* microfilaremic subjects were positive but not sera from subjects with *B. malayi* or with intestinal helminth infections, or normal control sera. Also sera from Myanmar migrants haboring the nocturnal periodic microfilariae of *W. bancrofti* were antigenemia positive (data not shown). Like ELISAs based on MAbs Og4C3 (More and Copeman, 1990) and AD12.1 (Weil *et al*, 1987) which specifically detect *W. bancrofti* circulating antigen in human sera, in this study ICT card test had no cross-reactivity. Our findings also had more conclusive evidence, of the 150 subjects whose stool samples were collected, 101 (67.3%) infected with intestinal helminths, mostly hookworms, *A. lumbricoides* and mixed infections (data not shown), were exposed to circulating filarial antigens. This is very important for there is a high prevalence of intestinal helminth infections in such *W. bancrofti*-endemic area in Tak Province. Although no possible explanation for the coexistence of filarial species other than *W. bancrofti* in the study area was debated, we cannot rule out the possibility of coexisting multiple filarial species in such endemic areas.

The sensitivity of the ICT card test was striking. When done with asymptomatic microfilaremia sera, it had a sensitivity of 99% using Og4C3 ELISA or AD12.1 ELISA as reference (Weil *et al*, 1997; Freedman *et al*, 1997). When done with endemic normal sera, it had a sensitivity of 65% when using AD12.1 ELISA and 100% when using Og4C3 ELISA (Weil *et al*, 1997). The AD12.1 ELISA and Og4C3 ELISA were double blind performed in different research laboratories using different sources of endemic normal sera kept in the serum banks. Such laboratory findings demonstrated that the ICT card test was less sensitive than AD12.1 ELISA for endemic normal sera with very low or borderline antigen levels (1-9 ng/ml) (Weil *et al*, 1997). It is very important that the ICT card test possibly had false-negative antigen test results in antigen-positive endemic normal sera. Prior population-based studies demonstrated the antigenemia prevalence rates in different endemic normal groups were 20% by Og4C3 filter paper test in India (Lalitha *et al*, 1998) and 16% and 17% by AD12.1 ELISA in India and Egypt, respectively (Weil *et al*, 1996). Twenty-eight (14%) of 205 endemic normals in Mae Chan villages of Tak Province were positive by the ICT card test but negative by microscopy and clinical and recall techniques, as we had no additional antigen testing for antigenemia level or provocative DEC and membrane filtration for microfilaria counts. In addition, we found that antigenemia in most endemic normal sera was detected within 15 minutes (as fast as 15 seconds were also observed), and some detected after 35 minutes (data not shown). These findings suggested, as similar to other ELISAs for early detection of the infection, the ICT card test could detect endemic normal individuals with *W. bancrofti* infection with a very low adult worm load.

The ICT card test had a sensitivity of 100% when done in microfilaremia sera using microscopy as reference. For microscopy the sensitivity of capillary tube technique was relatively similar to thick smear; as usually 60 µl blood samples analysed theoretically gave a very low detection limit of 15 microfilariae per ml. All microfilaremic samples either negative or positive by capillary tube technique were positive by thick smear. However, one was positive by capillary tube technique but negative by thick smear due possibly to technical errors. For the ICT card test, the sensitivities were the same when sera from subjects with both nocturnal

subperiodic and periodic *W. bancrofti* in the presence of very low number of the microfilariae were positive by the ICT card test (data not shown). Also it was not decreased when microfilaremia sera or plasma stored at -20°C for months were positive (data not shown). Such findings suggested that circulating antigen was truly not disappeared or degraded by the preparations and may be detected much longer afterwards.

The clinical and recall techniques used in this study was proposed for clinical disease survey of bancroftian filariasis in any cohorts of endemic population and essentially for the development of uniform physical examination for disease by local health worker. The ICT card test had a sensitivity of 84.6% when done in clinical disease sera using clinical and recall techniques as reference. Out of the clinical disease subjects, 3 with hydrocele (grade 2 of the extremity) were negative by microscopy and the ICT card test. Possible explanation for antigenemia undetected in sera from such clinical group is that those subjects habor dying or dead adult filarial worms (Ottesen *et al*, 1992). On the other hand, clinical and recall techniques was likely to be nonspecific for active infection. However, physical examination and "recall" questioning gave very good results. Eight of the 11 clinical disease subjects considered to have active clinical filariasis within a year were positive by the ICT card test. Of these 5 with most enlarged inguinal lymph node without retrograde inflammation of lymphatic tracks, were microfilremia positive by microscopy. Such findings suggested that uniform clinical and recall techniques could identify clinical disease subjects in endemic community for larger-scale morbidity control. However, technical skills of physical examiner supported by clinician was required. Taken altogether, such findings suggested that the ICT card test is diagnostic test for active *W. bancrofti* infections.

CFA detection by the ICT card test in serum can be done in ages, sexes, especially in people moving in to endemic area and in people given by DEC treatment under the field conditions in endemic areas. Furthermore, it may be suitable for determining infection prevalence for better estimate of bancroftian filariasis, especially when rapid assessment of the infections between migatory endemic carriers are required. Combination of clinical and recall techniques and the ICT card test may facilitate active surveillance, monitoring site-specific control strategies for bancroftian filariasis and identifying readily eradicated areas as proposed by WHO (Ottensen *et al*, 1995).

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