ICT FILARIASIS TEST : A NEW SCREENING TEST FOR BANCROFTIAN FILARIASIS

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Abstract. Bancroftian filariasis can be detected by using the ICT Filariasis test kit which is composed of specific polyclonal and monoclonal antibodies to *Wuchereria bancrofti* antigen. Chromatographic reaction with serum or plasma shows a result within 5 minutes. When compared with 454 thick blood films (standard smear method) within the same study, the ICT Filariasis test had sensitivity = 100%, specificity = 96.37%, efficiency = 96.70%, predictive value positive (PVP) = 70.70%, predictive value negative (PVN) = 100%. Compared with 454 membrane filtration technic (MFT), the MFT had sensitivity = 95.10%, specificity = 99.50%, efficiency = 99.12%, PVP = 95.10%, PVN = 99,50%. When we compared capillary tube technic (CAP) with TBF, CAP showed sensitivity = 85.40%, specificity = 100%, efficiency = 98.68%, PVP = 100%, PVN = 98.60%. With the convenience, high sensitivity-efficiency, lack of cross-reactions, no night blood collection, single reagent and rapidity of the test, the ICT Filariasis test can be recommended for screening of Bancroftian filariasis, and is suitable for the confirmation of suspected cases in the field where microscopic diagnosis is not availble.

INTRODUCTION

Bancroftian filariasis caused by nocturnally subperiodic *Wuchereria bancrofti* (rural strain) in Thailand is found along the Thai-Myanmar border from Mae Hong Son to Ranong Province since 1970 (Harinasuta *et al,* 1970; Sitthai, 1988; Phantana *et al,* 1996). The main strategy to control the disease is to reduce the microfilemia in human blood by prompt treatment. During 1993-1997, more than 700,000 Myanmar laborers came to work in Thailand. Surveys showed that about 2-6% of them were carriers of nocturnally periodic *Wuchereria bancrofti* (urban strain) (Phantana *et al,* 1996). The policy of the Ministry of Public Health, Thailand is to give mass drug administration (MDA), 6 mg/kg body weight of diethylcarbamazine citrated (DEC) 6 monthly, to reduce transmission of the disease in Thailand.

This control strategy faces practical problems : complete MDA coverage is not attainable because many laborers come to Thailand illegally thus avoiding government officers; they often migrate from place to place which usually provides a suitable environment for transmission; and the limitation of night blood collection for diagnosis and survey. Finger-prick blood film is still used for microfilaremia detection, but is time-consuming and not suitable for follow up. In this study we used the new screening test "ICT filariasis test" to screen the risk group and evaluated the sensitivity, specificity, efficiency, predictive value positive (PVP), predictive value negative (PVN).

MATERIALS AND METHODS

Study area : Mae Sot District of Tak Province is close to the Thai-Myanmar border and is endemic for Bancroftian filariasis and is about 490 km from Bangkok.

Study group : Myanmar laborers who come to work in Mae Sot District, Thailand.

Human blood : Night blood collection was made from 20.00 - 24.00 hours. Sixty microliters of finger pricked blood were drawn for thick blood film (TBF) by using hemoglobin pipette, dried, then stained with Giemsa solution (dilution 1 in 20 for 15 minutes), the parasites were examined and counted (Denham *et al,* 1971). Another 60 µl of blood were

placed in a capillary tube with heparin and centrifuged at 11,200 rpm for 5 minutes. The parasites were examined at the buffy coat (Pantana and Thammapalo, 1997; WHO, 1980).

One milliter of venous blood was drawn and kept in EDTA. The blood was filtered through a swin lock holder with polycarbonate membrane filter (diameter 25 mm, 5 μm pore size) by mixing 1 ml of blood with 9 ml of normal saline solution (NSS), slowly filtrated through the membrane, washed twice with 10 ml of NSS. A syringe of air was blown through the filter follwed by 4 ml of methanol. The membrane was removed from the holder, on a slide and stained with Giemsa (dilution 1 in 25 for 1 hour). After washing in tap water and then drying, parasites were counted by using 100X oil immersion objective. (Denis and Kean, 1971).

Human serum : Another 3 ml of blood were collected into a separate container, serum was separated after blood clotting and kept at -20°C until used.

Antigen detection with ICT Filariasis test kit : The test card was removed from the pouch just prior to use, opened and laid flat on the work surface. The adhesive on the right hand side of the test card was removed. 50 μl of sample serum was added on the pink pad, avoiding direct contact when adding the sample. Two drops of reagent A (included in the test kit set) were added onto the white pad indicated in the test card to ensure the pad was saturated. The bottle was held vertically when adding drops to ensure that the correct volume of buffer was dispensed and the card closed. To ensure good test flow, pressure was applied very firmly along the entire area to the right of the window and started timing. The result was read through the viewing window after 5 minutes. Low positive results may require up to 15 minutes to develop.

Interpretation of ICT test results

Positive : The test is positive if two lines (test and control) are seen in the viewing window. Any visible line in the test line area indicates a positive test result. The test is positive even when the test line appears lighter or darker than the control line.

Negative : The test is negative if only the control line is seen. To ensure that low positive samples have had sufficient time to develop, a negative result should not be recorded until 15 minutes have elapsed from when the card is closed.

Invalid : The test is invalid if the control line does not appear. If this occurs, the test should be repeated. Results should be read within 45 minutes of closing the card.

Cross-reaction : Infected serum of soil transmitted helminthiasis such as : *Necator americanus, Strongyloides stereo ralis, Ascaris lumbricoides, Trichuris trichiura, Brugia malayi* and normal human serum from endemic areas were examined with the ICT Filariasis test kit.

Statistics : The data were analysed by using Epi-Info version 6.02 for all experiments.

RESULTS

Night blood survey has been done on Myanmar laborers. A total of 454 samples were examined by thick blood film (TBF), capillary tube technic (CAP), membrane filtration technic (MFT) and ICT filariasis test and the number of positive cases and microfilarial positive rate were 41/9.03%, 35/7.71%, 41/9.03% and 58/12.77% respectively (Table 1).

When the TBF which is the standard technic was compared with ICT Filariasis test for sensitivity, specificity, efficiency, PVP and PVN, the results showed 100%, 96.37%, 96.70%, 70.70% and 100% respectively. When compared with MFT, the sensitivity was 95.10%, specificity = 99.50%, efficiency = 99.12%, PVP = 95.10% and 99.50%. When compared with CAP, the sensitivity was 85.40%, specificity = 100%, efficiency = 98.68%, PVP = 100% and PVN = 98.60% (Table 2).

The microfilarial density was classified in three levels as 1-10, 11-50, >50 mf/60 μl of TBF (Table 3). In this study we found 14 cases positive by ICT Filariasis test which were negative by TBF, CAP and MFT. These sera were confirmed as positive with ELISA test by using Mab Og4C3 from the ICT Diagnosis Laboratory, Australia (Table 4). Cross-reactions were examined with 12 soil transmitted helminthiasis sera, 2 cases from *Strongyloides stercolaris,* 2 from *Brugia malayi* microfilariae cases and 10 cases from non-endemic areas. There were no cross-reactions with ICT Filariasis test (Table 5). The advantages and disadvantages of the four technics are compared in Table 6.

Table 1

The nuber of patients and microfilarial positive rates (MPR) and antigen positive rates.

No. examined	Technic/no.of patient/MPR			
	CAP (%)	TBF (%)	MFT (%)	ICT (%)
454	35(7.71)	41(9.03)	41(9.03)	56(12.33)

Table 2

The sensitivity, specificity, predictive value positive, predictive value negative and efficiency of ICT[1]. MFT[2] and CAP[3] compared with TBF[4] (the standard technic).

Items	ICT%	95%CI	MFT %	95% CI	CAP %	95% CI
Sensitivity	100	89.30-100	95.10	82.20-99.20	85.40	70.10-93.90
Specificity	96.37	93.40-95.70	99.50	98.10-99.90	100	98.90-100
PVP[5]	70.70	57.10-81.50	95.10	82.20-99.20	100	87.70-100
PVN[6]	100	98.80-100	99.50	98.10-99.90	98.60	96.80-99.40
Efficiency	96.70	-	99.12	-	98.68	-

ICT[1] : Immunochromatographic test
MFT[2] : Membrane filtration technic
CAP[3] : Capillary tube technic
TBF[4] :Thick blood film
PVP[5] : Predictive value positive
VN[6] : Preditive value negative

Table 3

The number of positives in ICT according to density of microfilariae as determined by the thick blood film.

ICT	Density of microfilariae				
	0	1-10	11-50	>50	total
Positive	15	28	8	5	56

DISCUSSION

In this study it is clear that the ICF Filariasis test has high sensitivity, specificity and efficiency. For a chronic disease like filariasis, high sensitivity is more important than high specificity, and this test is useful for current control strategies (Suphantavanit, 1986). CAP technic showed lower sensitivity than ICT Filariasis test, confirming previous work by Sirichai *et al,* (1996). Results of the ELISA showed that the ICT Filariasis test has a high sensitivity and could detect low levels of antigen. The detection of specific bancroftian antigen instead of microfilare-

Table 4

Cross - check results (by ELISA) of serum samples that were positive by ICT/negative by thick blood film.

Patient no.	ICT	ELISA (Og4C3)
51	+	+(4+)
94	+	+(1/2+)
158	+	+(1+)
209	+	+(4+)
259	+	+(2+)
278	+	+(3+)
307	+	ND
363	+	+(3+)
371	+	+(4+)
379	+	+(3+)
404	+	+(3+)
425	+	+(3+)
434	+	+(3+)
437	+	+(1+)
453	+	+(2+)
203, 463	-	negative control
345	+	+(3+) positive control

ELISA (Og4C3) : ELISA test by ICT diagnostic New South Wales, Australia. ND : not done.

Table 5

The result of ICT Filariasis test against soil transmitted helminthiasis serum, *Brugia malayi* and normal serum (from non - endemic area).

Type of serum	No.	ICT results
1 *Necator americanus*	1	-
2 *Necator americanus* + *Ascaris lumbricoides* + *Trichuris trichiura*	2	-
3 *Ascaris lumbricoides* + *Trichuris trichiura*	6	-
4 *Trichuris trichiura*	3	-
5 *Strongyloides stercoralis*	2	-
6 *Brugia malayi*	2	-
7 Control from non - endemic area	10	-

Table 6

Comparison of the advantages and disadvantages of the four technics.

Result	ICT	MFT	CAP	TBF
1 Sensitivity	100%	95.10%	85.40%	Gold
2 Specificity	96.37%	99.50%	100.0%	Standard
3 Efficiency	96.70%	99.12%	98.68%	
4 Cost/case	55 Baht	50 Baht	10 Baht	10 Baht
5 Method of reading result	line of reaction	stain mf	live mf	stain mf
6 Rapidity	+	-	+	-
7 No microscope-microscopist required	+	-	-	-
8 Can use in field/remote area	+	-	+	+
9 Amicrofilaremia detection	+	-	-	-
10 Daytime blood survey	+	-	-	-
11 Long storage life	+	+	-	+
12 High MPR	+	-	-	-

Notes : 1 US$ = 37 Baht; + = acceptable; - = unacceptable

mia by using ICT Filariasis test is more convenient, a single reagent is used, the procedure is not complicated, the result is clear, it could be used in case of low microfilaremia and early detection (amicrofilaremia), lacks cross-reaction and the specimens can be collected during day time, no need of any provocative drug, no need of microscope and microscopist. With these advantages and the quality of the test, the ICT test can be recommended for screening people in bancroftian endemic areas to cut transmission and morbidity. The ICT Filariasis test should be an alternative test in the near future, although the cost is still high for developing countries. In future, we recommend that the test should detect both species of *W.bancrofti* and *B.malayi* in one test. The test should be adapted to use whole blood rather than serum. The test kit should be improved to withstand ambient temperature. The cost of the test should be reduced for developing countries.

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