Field evaluation of a rapid-format kit for the diagnosis of bancroftian filariasis in Egypt

R.M.R. Ramzy,[1] H. Helmy,[1] A.S.T. El-Lethy,[1] A.M. Kandil,[1] E.S. Ahmed,[1] G.J. Weil[2] and R. Faris[1]

تقييم ميداني لعتيدة سريعة الاستعمال في تشخيص داء الفيلاريات البنكروفية في مصر

رضا رمزي وحنان حلمي وأبو سريع الليثي وعمرو قنديل وإيهاب أحمد وجاري وايل ورفقي فارس

خلاصة: إن اختبار داء الفيلاريات المسمّى AMRAD-ICT (ICT-Fil) هو اختبار جديد سريع التطبيق على بطاقة لاكتشاف وجود المستضدات البنكروفتية في الدم البشري. ولقد قمنا بتقييم أداء هذا الاختبار في الظروف الميدانية في مصر، وذلك بمقارنة نتائج 1813 مشارك من منطقة متوطنة مع نتائج 102 مشارك من منطقة غير متوطنة. فتم فحص المجموعة الأولى بحثاً عن وجود المكروفيلاريات في الدورة الدموية (باللطاخة الثخينة والترشيح الغشائي) وبحثاً عن المستضدات في المصل. وبلغت معدلات العدوى التي تم التوصل إليها 2.8 % باللطاخة الثخينة، 3.5% بالترشيح الغشائي، 8.8 % باختبار إليزا، 9.0 % باختبار ICT-Fil. ولقد أمكن باختبار البطاقة اكتشاف المستضدات في الدورة الدموية في 98.0 % و95.3 % من حملة المكروفيلاريات في الدم، الذين كانت اختباراتهم إيجابية باللطاخة الثخينة وترشيح الدم على التوالي. أما المشاركون من المنطقة غير المتوطنة فقد كانوا سلبيين لاختبار ICT-Fil. وتم الحصول على نتائج مطابقة في حالة 173 من أصل 184 (94 %) من المشاركين من المنطقة المتوطنة، بعد اختبارهم بطريقة ICT-Fil المجهزة لفحص المصل أو لفحص الدم الكامل.

ABSTRACT The AMRAD-ICT Filariasis Test (ICT-Fil) is a new, rapid-format card test for the detection of bancroftian antigenaemia in human blood. We evaluated the performance of the test under field conditions in Egypt by comparing 1813 endemic and 102 nonendemic participants. Endemic participants were tested for microfilaraemia (thick smear and membrane filtration) and serum antigenaemia (ELISA). The infection rates detected were 2.8% by thick smear, 3.5% by membrane filtration, 8.8% by ELISA and 9.0% by ICT-Fil. The card test detected antigenaemia in 98.0% and 95.3% of microfilaraemia carriers testing positive by thick smear and blood filtration respectively. Nonendemic participants were ICT-Fil negative. Identical results were obtained for 173 out of 184 (94%) endemic participants tested by the serum and whole blood ICT-Fil versions.

Evaluation sur le terrain d'une trousse pour le diagnostic rapide de la filariose à Wuchereria bancrofti en Egypte

RESUME Le test AMRAD-ICT de diagnostic de la filariose (ICT-Fil) est un nouveau test rapide "sur carte" pour la détection des antigènes parasitaires dans le sang humain. Nous avons évalué les résultats de ce test dans la pratique sur le terrain en Egypte en comparant 1813 sujets provenant d'une zone d'endémie et 102 sujets d'une zone non endémique. Chez les sujets provenant d'une zone d'endémie, on a recherché la microfilarémie (goutte épaisse et filtration sur membrane) et l'antigénémie sérique. Les taux d'infection détectés étaient de 2,8% par goutte épaisse, 3,5% par filtration sur membrane, 8,8% par la méthode ELISA et 9,0% par le test ICT-Fil. Le test "sur carte" a détecté l'antigénémie chez 98,0% et 95,3% des sujets porteurs de microfiliaires dépistés positifs par goutte épaisse et par filtration sur membrane, respectivement. Les sujets provenant d'une zone non endémique ont eu des résultats négatifs au test ICT-Fil. Des résultats identiques ont été obtenus pour 173 des 184 (94%) sujets provenant d'une zone d'endémie qui ont été testés avec les versions ICT-Fil sang total et sérum.

[1]*Research and Training Centre on Vectors of Diseases, Ain Shams University, Cairo, Egypt.*
[2]*Washington University School of Medicine, St Louis, Missouri, United States of America.*
Received: 07/07/99: accepted: 30/09/99

Introduction

Nocturnally periodic bancroftian filariasis is endemic in several foci in the Nile delta in Egypt where it represents a major public health problem [*1,2*]. With the recent emergence of new, practical and cost-effective control measures, the World Health Organization (WHO) now considers the control and elimination of lymphatic filariasis from endemic areas, including those in Egypt, a reasonable and achievable goal. Identification of communities endemic with *Wuchereria bancrofti* infection is, therefore, of prime importance as the new WHO strategy for control programmes is for repeated, annual, mass therapy with single doses of diethylcarbamazine or combination regimens [*3*].

In Egypt, routine surveillance of nocturnally periodic filariasis, as in much of the world, relies on detection of microfilaraemia in thick blood films (20–50 µL). This method is relatively insensitive [*4*] and often impractical due to the necessity of blood collection at night. Although the sensitivity can be increased by membrane filtration of venous blood, people in endemic areas are often reluctant to comply.

Parasite antigen detection provides an alternative means to overcome the limitations associated with microfilaraemia detection and has the advantage of being able to detect amicrofilaraemic infections among "endemic normals". Previous studies by our group have shown that filarial antigen detection by a monoclonal (AD12) antibody-based, enzyme-linked immunosorbent assay (ELISA) is sensitive and specific for the diagnosis of active *W. bancrofti* infections [*5*]. Because this method allows parasite antigenaemia to be detected in finger-prick blood collected during the day, it provides a useful means for assessing filariasis endemicity in Egypt [*6*]. However, the system requires sophisticated equipment and cannot be performed directly in the field.

Recently, a filariasis card-kit test was developed by the Immunochromatographic Diagnostic Tests (AMRAD ICT) Company, Balgowlah, Australia. This ICT Filariasis (ICT-Fil) test is an antigen capture format that uses the AD12 monoclonal antibody and a polyclonal antibody attached to colloidal gold to detect filarial antigens in sera or plasma. To date, only one formal evaluation of this test has been performed; a laboratory study using well-characterized sera or plasma. The test was shown to be sensitive, specific, easy to perform and visually readable within minutes. However, as it requires sera separation, it cannot be executed in the field [*7*]. A new version of the filariasis card-kit test is now available based on the detection of circulating *W. bancrofti* antigens in finger-prick blood directly in the field and studies are needed to evaluate the performance of this new whole blood test in filariasis-endemic areas.

The aim of our study was to extensively determine the validity of the whole blood ICT-Fil card test under field conditions in Egypt. In particular, we compared the performance of this test with the traditional methods of microfilaria detection, in thick blood smears and filtration of venous blood, by assessing its sensitivity, specificity and positive and negative predictive values. In addition, we determined its capability to detect occult infections in "endemic normals", compared to antigen detection by ELISA.

Subjects and methods

The study was conducted in four villages in the Governerate of Al-Qalyubiya, located in an area known to be endemic with *W.*

bancrofti [6]. A house-to-house survey to locate bancroftian filariasis was carried out in the village of Kafr Tahoria and included all members of the household over 9 years of age. In the other villages, participants (9 years and over) were selected from a longitudinal study designed to follow a population subset, based on index children from Tahoria middle school which serves nearby villages [8]. After obtaining demographic information, household members were examined physically by at least one field physician from our team.

Four tests for the diagnosis of filariasis were performed on a total of 1813 endemic subjects (925 females and 888 males) (Table 1). Another 102 nonendemic control subjects residing in Cairo were tested by the ICT-Fil test and ELISA.

The ICT-Fil test was carried out according to the manufacturer's instructions. For this, a 100 µL finger-prick of blood was drawn onto the card and the results first read visually (negative/positive) after 15 minutes by three examiners. After being left to stand overnight at ambient conditions, the results were read again in order to observe any change. Unless the readings of all three examiners were in agreement, a sample was considered questionable and recorded as negative.

Blood samples for detection of microfilariae were obtained at night between 22.00 and 02.00. Thick blood smears (50 µL) were prepared from finger-prick blood samples and 1 mL of heparinized venous blood was filtered through membranes (5 µm pore size membranes from the Nuclepore Corporation, Pleasanton, California, USA). Thick films and membrane filters were Giemsa-stained and microscopically examined for the presence of microfilariae.

Filarial antigen was detected in plasma by AD12 monoclonal antibody-based ELISA as described by Ramzy et al. [5].

Table 1 **Age and sex distribution of participants in the endemic area**

Age group (years)	Female	Male	Total
10–20	467	466	933
21–30	142	109	251
31–40	161	143	304
41–50	86	112	198
51–60	42	38	80
> 60	27	20	47
Total	925	888	1813

The study was approved by Ain Shams University's institutional review board and informed consent was obtained from all participants. Data were analysed using *Epi-Info* version 6 and the chi-squared test was used to assess differences between categories. Sensitivity of the ICT-Fil test was defined as the ratio of those with a positive test divided by the number of microfilaraemic subjects by thick smear or membrane filtration. The negative and positive predictive values of the ICT-Fil test were determined using data obtained from microfilaraemia thick smears and membrane filtration as the gold standard tests.

Results

Diagnostic aspects of the ICT-Fil test

Of the 1813 participants studied using all four tests, 64 (3.5%) tested positive for microfilariae by membrane filtration. The mean microfilariae (MF) count ± standard deviation in these participants was 123 ± 158 MF/mL (range 1–755 MF/mL, median 81 MF/mL). Of these, 50 (78.1%) were also microfilaraemic by thick smear and 61 (95.3%) had positive ICT-Fil tests. In addi-

Table 2 **Sensitivity, specificity, positive and negative predictive values of the card test as compared to thick film and membrane filtration tests in participants in endemic areas**

Gold standard test	Whole blood card test					
	Positive (No.)	Negative (No.)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Thick smear			98.0	93.6	30.7	99.9
Positive	50	1				
Negative	113	1649				
Membrane filtration			95.3	94.2	37.4	99.8
Positive	61	3				
Negative	102	1647				

PPV = positive predictive value *NPV = negative predictive value*

tion, one case showed 1 MF by thick film examination and had a positive ICT-Fil test, but was free of microfilaria by membrane filtration. The mean microfilariae count ± standard deviation by thick smear was 13 ± 12 MF/50 µL (range 1–54 MF/50 µL, median 9 MF/50 µL). Of the 51 participants assessed as microfilaraemic by thick blood films, 50 (98.0%) were identified as ICT-Fil test positive. The ICT-Fil test had negative predictive values of 99.9% and 99.8% and positive predictive values of 30.7% and 37.4% (Table 2) when compared to thick smear and membrane filtration tests respectively.

Twenty participants (1.1%) from the whole study population had clinical filariasis (11 with lymphoedema, 6 with hydrocele and 3 with elephantiasis). All were amicrofilaraemic, and ICT-Fil- and ELISA-negative, except for two participants with hydrocele who had microfilariae (4 MF/mL and 65 MF/mL) and were ICT-Fil- and ELISA-positive. Consistent ICT-Fil test results were obtained when the cards were read after 15 minutes and again in the morning. Fifteen participants (0.8%) showed questionable ICT-Fil results and were recorded as negative. All were amicrofilaraemic and one had antigenaemia by ELISA. All 102 nonendemic participants were ICT-Fil-negative. A subset of 184 participants (representing 10% of the study population) were tested by both serum and whole blood ICT-Fil tests. Of these, 173 (94%) had matching results and 11 were positive with the whole blood ICT-Fil test only. Of these 11 participants, 4 had antigenaemia detected by ELISA, 1 of whom had 5 MF by membrane filtration.

Epidemiological aspects

The overall infection prevalence rates were 2.8% by thick smear, 3.5% by membrane filtration, 8.8% by ELISA and 9.0% by the ICT-Fil test. The prevalence rates of microfilaraemia by membrane filtration and antigenaemia (card test and ELISA) varied considerably by village (Table 3). This variation was statistically significant ($\chi^2 = 25.5$ and $\chi^2 = 16.4$ respectively, $P < 0.001$). The rank order of filarial antigenaemia in the four villages, as determined by the card test, correlated well with the results for microfilaraemia by membrane filtration (Table 3). Prevalence rates for microfilaraemia and

Table 3 **Prevalence of filarial infection in endemic villages, estimated by thick blood smear, membrane filtration, ELISA and ICT-Fil card-kit test**

Village		Positive by							
	No. examined	Blood film		Blood filter		ELISA		Card test	
		No.	%	No.	%	No.	%	No.	%
El-Quisom	630	6	1.0	11	1.7	34	5.4	52	8.3
Kafr Saad	151	1	0.7	3	2.0	26	17.2	1	7.3
Kafr Tahoria	851	31	3.6	33	3.9	64	7.5	69	8.1
Tahoria	181	13	7.2	17	9.4	35	19.3	31	17.1
Total	1813	51	2.8	64	3.5	159	8.8	163	9.0

ELISA = enzyme-linked immunosorbent assay

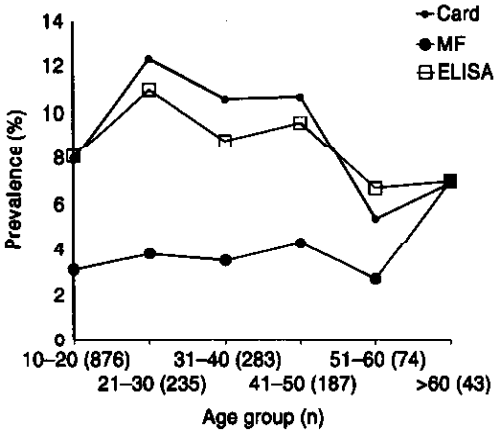

Figure 1 **Age prevalence profiles of participants with microfilaraemia detected by membrane filtration and antigenaemia by AD12-ELISA and ICT-Fil test. Numbers in parentheses indicate sample sizes of each age group**

antigenaemia did not vary with age or sex. Age-specific prevalence rates of microfilaraemia as determined by membrane filtration and antigenaemia by ICT-Fil test showed the same profile patterns (Figure 1).

Positive filarial antigen tests by ELISA were found in 86.3% and 86.0% of those testing positive for microfilaraemia by thick blood film and membrane filtration respectively. The ICT-Fil test detected 95.4% of microfilaraemic participants, as determined by either thick smear or membrane filtration, 50.5% of antigen-positive (by ELISA) amicrofilaraemic participants and 3.3% of antigen-negative (by ELISA) amicrofilaraemic participants (Table 4).

Discussion

The ICT-Fil test is a card-based test for the immunochromatographic detection of circulating *W. bancrofti* antigens. The first generation ICT-Fil test had excellent sensitivity and specificity, but the requirement for serum or plasma limited its application in the field [7]. The aim of our study was to evaluate the use of a new, whole blood version of the ICT-Fil test, as a simple, practical tool for the diagnosis of *W. bancrofti* infection in field conditions. This is the first field-based study to evaluate the performance of ICT-Fil in a filariasis-endemic area. Evaluation was based on rigorous comparison tests; membrane filtration of blood collected during the peak hours of

Table 4 **Filarial antigenaemia by the ICT-Fil test among endemic groups in villages in Qalubiya Governorate**

Village	Microfilaraemia carriers[a]		Antigen-positive[b] amicrofilaraemic participants		Antigen-negative[b] amicrofilaraemic participants	
	No.	%	No.	%	No.	%
El-Qulsom	11/11	100	14/25	56.0	26/594	4.4
Kafr Saad	3/3	100	5/22	22.7	3/126	2.4
Kafr Tahoria	33/34	97.0	21/37	56.7	21/781	2.7
Tahoria	15/17	88.2	12/19	63.1	4/145	2.8
Total	62/65	95.4	52/103	50.5	54/1646	3.3

[a] *Microfilaraemia determined by either thick smear or membrane filtration*
[b] *Detected by ELISA*

microfilaraemia was used as the gold standard, plus microfilarial detection by thick blood smears, as this is the method currently being used for routine surveys. Results from the study showed that the ICT-Fil test successfully identified 98% of microfilaraemic participants detected by thick smear and 95.3% identified by membrane filtration, whereas nonendemic control subjects were uniformly negative. This shows that the ICT-Fil test is highly sensitive and specific, and is highly recommended as a replacement for the microfilaria detection tests currently used in the routine surveillance of bancroftian filariasis.

It is of special interest that, although the card test showed higher village-specific filarial prevalence rates compared to microfilaria detection, both methods provided the same ranking for the four villages. The same observation was true for age-specific prevalence rates, which exhibited similar profiles (Figure 1) and these speak well for the validity of the card test.

The ICT-Fil test had excellent negative predictive values (99.9% and 99.8%), but its positive predictive values were low (30.7% and 37.4%) when compared to microfilaria detection by thick smear and membrane filtration respectively. These low positive predictive values are mainly due to the fact that the ICT-Fil test detects antigenaemia in amicrofilaraemic people. Several studies have demonstrated that amicrofilaraemic individuals with positive antigen tests are actually infected with *W. bancrofti* [*3,5,9*]. Thus, if we calculate the positive predictive value of the card test based on filarial infection to include those with positive tests for MF by thick film or membrane filter or antigen by ELISA (Table 4), the positive predictive value would increase dramatically to 67.8% (114 out of 168).

Although the overall filaria prevalence rates for the ICT-Fil test and the AD12-ELISA were fairly close (8.8% and 9.0% respectively), these tests did not detect the same antigen-positive endemic normal individuals. This could be attributed to the different detection systems used in these assays; polyclonal antibody attached to colloidal gold in the case of ICT-Fil test and peroxidase conjugated to AD12 monoclonal antibody for ELISA. A previous

study reported similar observations, where 86% and 46% of antigen-positive endemic normal individuals with relatively high and low antigen levels by ELISA, respectively, were ICT-Fil-test positive [7]. In this study, however, antigen levels were not quantitatively measured.

The whole blood version of the ICT-Fil test showed slightly higher sensitivity than the serum version, perhaps due to the larger serum volume (about 65 µL) contained in the 100 µL blood sample compared to 50 µL used in the first generation (serum or plasma) ICT-Fil test.

The second generation whole blood ICT-Fil test has tremendous technical and practical advantages over microfilaria detection for routine surveys. It can be performed during the day and results are obtained quickly, directly in the field. The test does not require any clinical laboratory facilities or experienced microscopists. Because less than 1% of the ICT-Fil cards were questionable, this indicates that, given adequate training, the test can be performed and easily read by individuals in rural clinic settings. The near instantaneous read-out of the whole blood ICT-Fil test has made it a promising new tool which we found capable of improving community compliance.

WHO is advocating new strategies for the control and elimination of lymphatic filariasis. These are based on the distribution of rounds (repeated according to disease endemicity) of a community-wide, single annual dose of combined drug regimens. One essential element for the success of such control efforts is the identification and assessment of filariasis endemicity in affected communities. We firmly believe that the whole blood ICT-Fil test will play a major role in this task.

Acknowledgements

This work was partially supported by the WHO Regional Office for the Eastern Mediterranean, grant SGS97/8 and the National Institute of Health, grant AI-35855. Excellent technical assistance was provided by Ms Madiha Shaban and Mr Rawhy Gergeous.

References

1. Harb M et al. The resurgence of lymphatic filariasis in the Nile delta. *Bulletin of the World Health Organization*, 1993, 71(1):49–54.

2. Faris R et al. Community diagnosis of bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1993, 87(6):659–61.

3. Ottesen EA et al. Strategies and tools for the control/elimination of lymphatic filariasis. *Bulletin of the World Health Organization*, 1997, 75(6):491–503.

4. *WHO Expert Committee on Filariasis. Lymphatic filariasis. Fourth Report of the WHO Expert Committee on Filariasis.* Geneva, World Health Organization, 1984. (Technical Report Series, No. 702).

5. Ramzy RMR et al. Evaluation of a monoclonal-antibody based antigen assay for diagnosis of *Wuchereria bancrofti* infection in Egypt. *American journal of tropical medicine and hygiene*, 1991, 44(6): 691–5.

6. Ramzy RMR et al. Efficient assessment of filariasis endemicity by screening for filaria antigenaemia in a sentinel population. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88(1):41–4.

7. Weil GJ et al. The ICT-Fil filariasis test: a rapid-format antigen test for diagnosis of bancroftian filariasis. *Parasitology today*, 1997, 13:401–4.

8. Weil GJ et al. A longitudinal study of bancroftian filariasis in the Nile delta of Egypt: baseline data and one year follow-up. *American journal of tropical medicine and hygiene*, 1999, 61(1):53–8.

9. Weil GJ et al. Parasite antigenemia without microfilaremia in bancroftian filariasis. *American journal of tropical medicine and hygiene*, 1996, 55(3): 333–7.

Partnerships for long-term goals

Seven infectious diseases — filariasis, leprosy, guinea-worm disease, tetanus, Chagas disease, measles and poliomyelitis have been targeted by WHO for eradication/elimination. WHO's immediate aim is to lower their prevalence so that they are more easily managed by health systems.

Effective public/private sector partnerships are exemplified in WHO's Eradiacation and Elimination Programme against these seven diseases. Like poliomyelitis, the eradication of guinea-worm disease is in its final phase with a stubborn, but disappearing incidence in only 14 of the initial 20 countries where the disease was rampant at the beginning of the campaign.

Diseases such as leprosy and lymphatic filariasis, Chagas disease and measles are being eliminated, with the disease burden decreasing dramatically. Partnerships include public and private organizations and institutions, NGOs and the pharmaceutical industry.

Information on Eradication and Elimination Programmes is found at www.who.int/ctd and www.who.int/lep and www.filariasis.org/index

Source: Removing obstacles to healthy development: report on infectious diseases. *Geneva, World Health Organization, 1999:25.*