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The ICT Filariasis Test: A Rapid-format Antigen Test for Diagnosis of Bancroftian Filariasis

G.J. Weil, P.J. Lammie and N. Weiss

Antigen testing is now recognized as the method of choice for detection of Wuchereria bancrofti *infections. Unlike tests that detect microfilariae, antigen tests can be performed with blood collected during the day or night. However, existing enzyme-linked immunosorbent assay (ELISA) tests for filarial antigenemia are difficult to perform in the field, and this has limited their use in endemic countries. In this article, Gary Weil, Patrick Lammie and Niggi Weiss review their experience with a new rapid-format filarial antigen test. They found that the ICT card test was very easy to perform and that it was comparable with ELISA for the detection of filarial antigen in sera from people with microfilaremia. The introduction now of an antigen test suitable for use in the field is especially timely, in that it may facilitate implementation of new strategies proposed by the World Health Organization for control and elimination of lymphatic filariasis.*

Lymphatic filariasis is a deforming parasitic disease that affects over 100 million people in more than 70 tropical and subtropical countries[1,2]. Most lymphatic filariasis is caused by the mosquito-borne nematode, *Wuchereria bancrofti*. Infections may be asymptomatic, but often they are associated with acute syndromes such as lymphangitis with fever and with chronic complications such as hydroceles in men and the most dreaded outcome of infection, physically and socially disabling elephantiasis of the extremities.

Traditional control programs for lymphatic filariasis have relied primarily on antimosquito measures and on administration of diethylcarbamazine (DEC) therapy to infected and/or diseased persons identified in active screening programs. While filariasis has been controlled and even eliminated in some areas with these methods, control efforts in many settings have only stabilized infection rates or lost ground. However, new tools for diagnosis and therapy of filariasis and new control strategies have recently re-energized the struggle against filariasis[1–3] and, finally, the tide may be turning against this difficult disease.

Diagnostic options for bancroftian filariasis

Efficient diagnosis of *W. bancrofti* infection is especially important as control programs move towards the new strategy of community diagnosis and repeated, annual mass therapy with single-dose DEC or combination regimens[1–3]. Several methods are available for diagnosis of bancroftian filariasis (Table 1). Clinical diagnosis is labor-intensive, insensitive and not specific for active infection. Parasitological methods for diagnosis depend on detection of microfilariae (Mf) in peripheral blood. The sensitivity of Mf detection depends on the volume of blood sampled, the time of blood collection (because of nocturnal periodicity of microfilaremia in most endemic areas) and the skill and dedication of the microscopist. Microscopy-based diagnostic methods[4], while time-honored and 'low-tech', are relatively difficult procedures to

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  0169-4758/97/$17.00 S0169-4758(97)01130-7

Table 1. Diagnostic options for detection of *Wuchereria bancrofti* infections[a]

Method	Sensitivity	Specificity	Technical difficulty	Cost
Clinical examination	+	++	++	NA[b]
Mf thick smears	++	+++	++[c]	+
Mf membrane filtration	+++	+++	+++[c]	++
DNA detection (PCR)	+++	++++	++–+[c]	++++
Antibody serology	+++	++	+++	+++
Antigen, ELISA	++++	++++	+++	+++
Antigen, ICT card test	++++	++++	+	++

[a] NB Semiquantitative ratings/opinions are based on the personal experiences of the authors, with '+' lowest and '++++' highest. Cost ratings are based on the cost of materials and requirements for expensive laboratory equipment.
[b] No score assigned; highly variable.
[c] Mf detection requires night blood samples in areas where Mf exhibit nocturnal periodicity. Additional testing is needed to determine the sensitivity of PCR for detection of parasite DNA in blood collected by day in areas with nocturnally periodic Mf. Preliminary results have shown that PCR can detect parasite DNA in some day blood samples, but signals are stronger with night blood.

perform well and with consistency in field situations. The thick smear is the simplest Mf detection method and the most widely used in the field. Thick blood films (typically 20–60 μl) are stained and examined for Mf by microscopy. Membrane filtration of venous blood is more sensitive for detecting Mf than examination of thick smears, but the cost of materials (syringes and filters) and amount of technician time associated with this method are high and preclude its routine use in most endemic areas.

Problems associated with Mf detection have led to development of alternative methods for the diagnosis of filariasis including detection of antibodies to the parasite, detection of parasite DNA by the polymerase chain reaction (PCR) and immunological detection of soluble parasite antigens in human blood. New antibody tests based on recombinant antigens are reasonably sensitive and more specific than earlier tests based on native filarial antigens[5,6], but antibody tests do not distinguish between current and past infections, and antibody titers are not correlated with infection intensity, ie. the number of worms in the patient.

Recent studies have shown that filarial DNA can be detected in human blood (and sputum!) by PCR[7,8]. Laboratory evaluations have shown that DNA detection can be very sensitive for the diagnosis of people with circulating Mf, and can provide a species-specific diagnosis. However, the very sensitivity of PCR tests can be a limitation, because special care is needed to prevent cross-contamination of specimens and false-positive results. Like membrane filtration, parasite DNA detection by PCR is highly labor-intensive and expensive for routine use in endemic areas.

Detection of circulating filarial antigens is a useful non-microscopic method for diagnosis of bancroftian filariasis that is currently making the transition from the research laboratory to the field. Several groups have reported detection of soluble filarial antigens in human blood by sensitive immunological techniques including immunoradiometric assay and enzyme-linked immunosorbent assay (ELISA)[9–12]. Extensive studies have shown that some filarial antigen tests are more sensitive than thick smear or membrane filtration for detection of *W. bancrofti* infections, and that they provide a species-specific diagnosis; sera from people infected with closely related parasites (*Brugia malayi*, *Onchocerca volvulus* and *Loa loa*) are negative in these tests. Antigen tests sometimes detect filarial antigens in sera from people in endemic areas who are amicrofilaremic and asymptomatic. Recent studies suggest that these test results are not artifacts and that antigen-positive endemic normals harbor mature filarial infections[13]. Unlike membrane filtration, antigen tests can be performed with finger-prick blood specimens collected during the day or night. Animal studies have shown that parasite antigen levels provide an indication of adult worm infection intensity in filariasis[14,15], and antigen testing has been used to monitor the efficacy of therapies against adult filarial worms *in vivo*[16,17]. Although serum filarial antigen levels often decrease after humans are treated with DEC and/or ivermectin, antigen tests usually remain positive months after therapy with current regimens, even in cases where Mf are persistently cleared from the blood[18].

All tests have limitations, and this also applies to antigen testing for filariasis. One limitation is that filarial antigen testing is not sensitive for confirmatory testing of patients suspected of having clinical filariasis. Many patients with clinical filariasis are amicrofilaremic, and most of these people have negative serum antigen tests. These negative Mf and antigen tests suggest that many people with chronic clinical filariasis do not harbor live adult worms. A second limitation of filarial antigen tests has been that they are not practical for routine use in most endemic areas. The Gib-13 immunoradiometric assay[9] and enzyme immunoassays based on monoclonal antibodies AD12.1 (Ref. 10) and ES-33 (Ref. 19) are all research tests that are not commercially available. One company has marketed a *W. bancrofti* antigen test kit based on the monoclonal antibody Og4C3 (Trop-Ag *W. bancrofti*, JCU Tropical Biotechnology Pty Ltd, Townsville, Queensland, Australia). This test has been well received in the research community[20,21] and the authors have found it to be sensitive and reliable. The main limitations of this test are its expense and format. Leaving aside the issue of cost, the double sandwich ELISA format is somewhat cumbersome and labor-intensive, and the test requires a level of laboratory infrastructure for performance and interpretation of results that has prevented its wide acceptance in endemic areas.

The ICT Filariasis card test

The ICT Filariasis test is a new, rapid-format filarial antigen test that was developed by ICT Diagnostics (Balgowlah, New South Wales, Australia). The test detects soluble *W. bancrofti* antigens that circulate in the blood of infected humans[22]. Human serum or plasma (without pretreatment) is added to a pink sample pad that contains dried polyclonal antifilarial antibodies coupled to colloidal gold (Fig. 1). Two drops

of wash buffer (Reagent A) are then added to a separate wash pad. Labeled antibody and filarial antigen (if present) flow towards the top of the card across a nitrocellulose strip when the cardboard kit is closed. A monoclonal antifilarial antibody (AD12.1; Ref. 22) bound to the nitrocellulose traps free antigen and antigen–antibody complexes and concentrates the gold-labeled conjugate to form a visible pink line. Most positive sera can be identified in less than 5 min, but the sensitivity of the test is slightly improved if it is read after 15 min. A procedural control is incorporated in each test.

Test evaluation. The ICT Filariasis card test was evaluated independently in three laboratories (Weil, Washington University, St Louis, MO, USA; Weiss, Swiss Tropical Institute, Basle, Switzerland; Lammie, Centers for Disease Control and Prevention, Atlanta, GA, USA) using well-characterized filariasis sera from Egypt and India (Weil), the WHO Filariasis Serum Bank (sera from many different endemic areas) (Weiss) and Haiti (Lammie). Non-endemic US sera tested at Washington University (including sera with high titers of rheumatoid factor and antinuclear antibodies) were a gift from the Clinical Immunology Laboratory at Barnes-Jewish Hospital, St Louis, MO, USA. All sera were tested blindly. All sera were tested also for filarial antigenemia by ELISA with the Trop-Ag test (Lammie and Weiss) or with a non-commercial ELISA based on monoclonal antibodies DH6.5 and AD12.1 (Ref. 10).

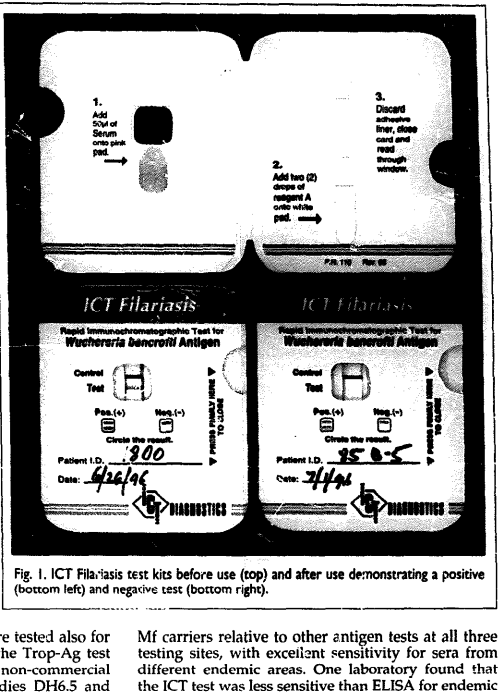

Fig. 1. ICT Filariasis test kits before use (top) and after use demonstrating a positive (bottom left) and negative test (bottom right).

Test results are presented in Table 2. The ICT Filariasis test performed nearly perfectly with sera from Mf carriers relative to other antigen tests at all three testing sites, with excellent sensitivity for sera from different endemic areas. One laboratory found that the ICT test was less sensitive than ELISA for endemic normal sera with very low or borderline antigen levels (1–9 ng ml^{-1}). False-positive results were not observed with non-endemic sera, including sera with high titers

Table 2. ICT Filariasis antigen card test performance

	Serum source[a]				
	Wuchereria bancrofti endemic				
Laboratory	**Mf$^+$**	**Mf$^-$, Ag$^+$**	**Mf$^-$, Ag$^-$**	**Other parasites**	**Non-endemic**
St Louis (Weil)[b]	51/53 (96%)	30/46 (65%)	3/48 (6%)	1/23 (4%)	0/70 (0%)
Basle (Weiss)[c]	48/48 (100%)			0/50 (0%)	0/20 (0%)
CDC (Lammie)[d]	32/32 (100%)	23/23 (100%)	0/53 (0%)		0/11 (0%)

[a] Data shown are the number of sera positive in the ICT test, the total number tested and the % positive.
[b] Other parasites sera included ten onchocerciasis sera from Nigeria and 13 brugian filariasis sera from India (all Mf positive). One antigen-positive *Brugia* serum was from an area co-endemic for *W. bancrofti* in S. India. This serum was also positive in the Trop-Ag and AD12.1 antigen assays. Non-endemic sera included 20 with positive rheumatoid factor (titers ≥1:160) and ten with antinuclear antibodies. Three of 48 sera from antigen-negative endemic normals (Mf negative and antigen negative by the AD12.1 assay) were positive in the ICT Filariasis test. Antigen positivity in these three sera was confirmed by the Trop-Ag test. The ICT test was positive in 19/22 (86%) sera from endemic normals with antigen levels ≥10 ng ml^{-1} by AD12.1 ELISA. Only 11/24 (46%) of sera with very low or borderline antigen levels (2–9 ng ml^{-1}) were positive in the ICT test.
[c] Other parasites sera were from 20 patients with onchocerciasis, five with *Mansonella perstans*, five with *Loa loa*, five with strongyloidiasis, five with echinococcosis and ten with various protozoan infections.
[d] Complete agreement with Trop-Ag kit for 108 sera from Haiti. This included 32 people with microfilaremia, 23 Mf-negative people with positive Trop-Ag antigen tests and 53 Mf-negative people with negative Trop-Ag antigen tests.

of rheumatoid factor and antinuclear antibodies. One apparent false-positive serum from a patient with brugian filariasis came from an area in Kerala, South India, where dual infections with *B. malayi* and *W. bancrofti* are sometimes seen. This serum was also positive in the AD12.1 and Trop-Ag assays, while all other sera from patients with brugian filariasis were negative by all three antigen tests.

Significance. The ICT Filariasis test has a number of advantages over filarial antigen tests described previously. It is the first rapid-format antigen test to be introduced for filariasis and it is very easy to perform. The test can be performed under true field conditions, as it does not require pretreatment of sera or special equipment. The tests are read visually, and the cards can be labeled and stored as permanent records. Colloidal-gold antibody conjugates should be more stable under field conditions than enzyme–antibody conjugates and substrates. The test would be even more useful if it could be performed with whole blood collected by finger-prick, and work to modify the test for whole blood testing is in progress (G. Mearns, ICT Diagnostics, pers. commun.).

This test may prove to be a very useful new tool in the global effort to control and eliminate lymphatic filariasis because it can be performed in all endemic areas, even under primitive field conditions. The test will find some use in clinics and hospitals for diagnosis of individual patients, but its greatest value is likely to be for primary surveillance to assess filariasis infection prevalence rates in populations prior to mass therapy. Field workers will now be able to diagnose filarial infections with finger-prick blood specimens collected during the day or night with no need for a laboratory or even a microscope. The near-immediate readout may enhance community compliance for surveillance and mass-therapy programs. The test should also prove useful for detection of early infections in children or other sentinel populations who can be tested as a means of assessing the success of control programs designed to interrupt filariasis transmission.

Acknowledgements

Barnes-Jewish Hospital has licensed materials to ICT Diagnostics for use in the ICT Filariasis test kit. ICT Diagnostics provided free kits to all three laboratories for evaluation without payment for testing and without restrictions on the data obtained. Weil's laboratory is supported in part by the National Institutes of Health and WHO/TDR. Fanya Liftis provided excellent technical support. Lammie's work in Haiti was supported in part by grants from WHO/TDR and USAID. Mark Eberhard and David Addiss participated in studies in Haiti that generated serum samples tested in this study. The Filariasis Serum Bank was supported in part by TDR/WHO. Annelise Oettl provided excellent technical support in Basle.

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