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EVALUATION OF ICT FILARIASIS CARD TEST USING WHOLE CAPILLARY BLOOD: COMPARISON WITH KNOTT'S CONCENTRATION AND COUNTING CHAMBER METHODS

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ABSTRACT: An immunochromatographic card test (ICT) that uses fingerprick whole blood instead of serum for diagnosis of bancroftian filariasis has recently been developed. The card test was validated in the field in Kenya by comparing its sensitivity to the combined sensitivity of Knott's concentration and counting chamber methods. A total of 102 (14.6%) and 117 (16.7%) persons was found to be microfilaremic by Knott's concentration and counting chamber methods, respectively. The geometric mean intensities (GMI) were 74.6 microfilariae (mf)/ml and 256.5 mf/ml by Knott's concentration and counting chamber methods, respectively. All infected individuals detected by both Knott's concentration and counting chamber methods were also antigen positive by the ICT filariasis card test (100% sensitivity). Further, of 97 parasitologically amicrofilaremic persons, 24 (24.7%) were antigen positive by the ICT. The overall prevalence of antigenemia was 37.3%. Of 100 nonendemic area control persons, none was found to be filarial antigen positive (100% specificity). The results show that the new version of the ICT filariasis card test is a simple, sensitive, specific, and rapid test that is convenient in field settings.

Currently used parasitological diagnostic procedures for bancroftian filariasis include membrane (Nuclepore) filtration, stained thick smear, Knott's concentration, and counting chamber methods. All require night-blood sampling to detect the nocturnally periodic *Wuchereria bancrofti* microfilaremia. Nighttime blood sampling is cumbersome and inconvenient for both investigators and endemic communities, resulting in low compliance. This constraint has prompted an extensive search for assays for bancroftian filariasis that are not affected by the periodicity of microfilariae (mf) in blood.

Various antibody-based assays have been developed to detect host antibodies against *W. bancrofti*, and specific IgG4 has been shown to be the best marker for active infection (Lal and Ottesen, 1988; Kwan-Lim et al., 1990). However, the major limitation of antibody-based assays is that they cannot distinguish between past and current infections. Several assays based on detection of circulating filarial antigen (CFA) have been developed in the recent past and are promising tools for diagnosis and control of bancroftian filariasis. Positive CFA test results in sera from endemic persons indicate the presence of active *W. bancrofti* infection (Weil et al., 1996). One such CFA-based assay is the highly specific and sensitive monoclonal antibody-based Og4C3 assay (More and Copeman, 1990). The Og4C3 assay is a monoclonal-based enzyme-linked immunosorbent assay (ELISA) that specifically detects CFA in sera from patients with *W. bancrofti* infection. However, the assay is not easy to use in field settings because it requires specific equipment, such as an ELISA reader.

Polymerase chain reaction (PCR) assays have been developed for species-specific detection of filarial parasites in blood samples from infected individuals (McCarthy et al., 1996; Williams et al., 1996). However, PCR assays require sophisticated equipment and significant expertise, and are difficult to adapt for field settings because the protocols are rather stringent.

ICT Diagnostics (Balgowlah, Australia) has developed a specific, rapid immunochromatographic card test (ICT) for detecting *W. bancrofti* circulating antigen in serum or plasma (Weil et al., 1997). The ICT filariasis card test detects CFA released by adult worms (Weil et al., 1996), and can therefore diagnose

adult worm infections independent of microfilaremia (Simonsen and Dunyo, 1999). Recently, a new version of the ICT card test that detects CFA in whole blood has been developed. The advantages of the ICT filariasis card test over other antigen-based tests are that it is easy to use in the field and can use fingerprick daytime blood samples, which are more acceptable to the endemic communities. Other advantages are that the methodology needs minimal expertise and there is no equipment requirement.

The present study evaluated the suitability of the new version of ICT filariasis card test using fingerprick whole blood for diagnosis of *W. bancrofti* infection in field setting and compared it with the Knott's concentration and counting chamber methods. The validity of the ICT filariasis card test was based on the assessment of its sensitivity and specificity.

MATERIALS AND METHODS

Study areas

The ICT filariasis card test was compared with the Knott's concentration and counting chamber methods in Muhaka, a filariasis-endemic area in Kwale District, Coast Province, Kenya (Wamae et al., 1998). Negative controls were persons from Kimende, a high-altitude nonendemic area in Kiambu District, Central Province, Kenya.

Selection of study groups

A total of 701 persons from Muhaka was screened for microfilaremia by the counting chamber method. Of these 701 persons, 697 were also tested for microfilaremia by the Knott's concentration method. The remaining 4 persons refused venipuncture.

Ninety-six microfilaremic persons (positive by both counting chamber and Knott's concentration methods) were enrolled as the endemic microfilaremic group. Similarly, 97 amicrofilaremic persons (by both parasitological methods) were enrolled as the endemic amicrofilaremic group.

A total of 100 individuals from Kimende was enrolled as the nonendemic negative controls. Only persons who were born and resided all their lives in the nonendemic area were enrolled.

Blood sampling

Nighttime (between 2100 hr and 2400 hr) 2–3-ml blood samples were collected by venipuncture into 5-ml Vacutainer tubes containing EDTA. Additionally, 100 µl of fingerprick blood samples were collected using heparinized capillary tubes and immediately mixed with 900 µl of 3% acetic acid in 10-ml polystyrene tubes. The specimens were stored at ambient temperature until the following morning.

Parasitologic tests

On the following morning, the venous blood specimens were carefully resuspended and 1 ml of each sample mixed with 10 ml of 2%

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TABLE I. Prevalence and intensity (GMI) of *Wuchereria bancrofti* microfilaremia by counting chamber and Knott's concentration methods.

Method	n	No. pos. (%)	GMI (mf/ml)	Range
Counting chamber	701	117 (16.7)	256.5	1–930
Knott's concentration	697*	102 (14.6)	74.6	1–1881

* Four persons refused venipuncture.

formalin in 15-ml centrifuge tubes for the Knott's concentration method (Knott, 1939). The contents were mixed thoroughly by shaking and allowed to stand for 15 min before centrifugation at 450 g for 5 min. The supernatant was poured off and the sediment used to make a wet smear on a microscope glass slide. The smears were examined for the presence of mf under a binocular microscope. The remaining venous blood specimens were used for plasma separation. The plasma specimens were used for immunological assays in a related study to be reported elsewhere.

The counting chamber method was performed as previously described (McMahon et al., 1979). Blood specimens in 3% acetic acid were transferred into a counting chamber and the mf counted under a binocular microscope.

In both methods, mf counts were expressed as mf/ml blood. Geometric mean intensity (GMI) of microfilaremia was calculated as $\text{antilog}[\sum \log(x + 1)/n]$, where x is the number of mf/ml blood from mf-positive persons and n the number of mf-positive persons examined.

The ICT filariasis card test

The whole-blood version of the ICT filariasis card test (ICT Diagnostics, catalogue no. ICT FL02) was performed according to the manufacturer's instructions supplied with the test kit. Briefly, 100 μ l of fingerprick daytime blood samples were collected using heparinized capillary tubes and immediately applied dropwise to the white area of the pink and white pad. After the pink area of the pad was completely wet with plasma, the card was closed and results read within 15 min. All specimens rated negative after 15 min were reexamined after 1 hr for confirmation.

Validation of the ICT filariasis card test

The sensitivity of the ICT filariasis card test was defined as its ability to detect CFA in parasitologically confirmed (by both Knott's concentration and counting chamber methods) mf-positive persons. The specificity of the card test was defined as its ability to exclude persons not infected with *W. bancrofti* and was estimated by computing the percentage of nonendemic persons who had a negative card test reading. Additionally, 97 mf-negative persons from the endemic area were tested to determine additional sensitivity of the ICT filariasis card test, thereby picking up false negatives by the standard parasitological tests.

RESULTS

A total of 117 (16.7%) persons was found to be microfilaremic by the counting chamber method compared with 102 (14.6%) persons by Knott's concentration method (Table I). The GMIs were 256.5 mf/ml by the counting chamber method compared with 74.6 mf/ml by Knott's concentration method ($P < 0.0001$).

Only persons who were mf-positive by both methods were selected for the sensitivity testing. The characteristics of the study groups are shown in Table II. All parasitologically confirmed microfilaremic persons ($n = 96$) were also antigen positive by the ICT filariasis card test, that is, the sensitivity of the card test was 100%. Further, of 97 parasitologically amicrofilaremic persons, 24 (24.7%) were antigen positive by the ICT filariasis card test. Of 100 persons from the nonendemic area,

TABLE II. Characteristics of the study groups by immunochromatographic filariasis card test (ICT) results.

Group	n	Avg. age (range) in years	Sex (M/F)	No. pos. by ICT (%)
Endemic, microfilaremic	96	31.7 (9–80)	62/34	96 (100.0)
Endemic, amicrofilaremic	97	31.2 (8–99)	64/33	24 (24.7)
Nonendemic controls	100	42.6 (10–94)	69/31	0 (0.0)

none was filarial antigen positive (100% specificity). In all the card tests performed, a reading was possible within 5 min and did not change when reexamined after 1 hr.

The true prevalence of *W. bancrofti* infection in the area, on the basis of the counting chamber method and the ICT filariasis card test, was calculated as follows: $16.7\% + [24.7\% \times (100 - 16.7)] = 37.3\%$.

DISCUSSION

Recent research advances in lymphatic filariasis have led to new understanding about the severity and impact of the disease, new diagnostic and monitoring tools, and, most importantly, new treatment and control strategies (Ottesen et al., 1997). In 1993, an International Task Force for Disease Eradication identified lymphatic filariasis as 1 of only 6 eradicable, or potentially eradicable, infectious diseases (CDC, 1993). On the basis of these advances, in 1997, the 50th World Health Assembly launched an initiative to eliminate lymphatic filariasis globally as a public health problem (WHO, 1997) and, toward this goal, many endemic countries are now developing national programs and plans of action.

The present study demonstrates that both the sensitivity and specificity of the ICT filariasis card test using daytime whole-blood specimens are 100%. *Wuchereria bancrofti* is the only known filarial parasite in the population, although other helminths, such as intestinal worms and *Schistosoma haematobium*, are also endemic. Although the card test has the limitation of giving only a qualitative (positive/negative) result, its degree of sensitivity and specificity is expected to be useful in identifying endemic communities for treatment in the lymphatic filariasis elimination programs and certifying elimination thereof. The unit price of the card test (around \$1.30 [U.S.]) may raise doubts about its extensive field application. However, several factors, such as use of fingerprick whole-blood specimens, ease of performance, daytime application, and shorter time to obtain results make the whole-blood version of the ICT filariasis card test convenient in field settings.

A study in South India (Pani et al., 2000) compared the whole-blood-based ICT test with both thick blood smear and membrane filtration methods. Although the specificity of the ICT test was 100% in comparison with both parasitological tests, the sensitivity was 98.5% against the fingerprick thick blood smear and 71.9% against the membrane filtration method. However, the ICT test failed to detect CFA in 20 of 30 (67%) individuals with mf count less than 10 mf/ml. This result contradicts the observation that the ICT test, in addition to identifying all microfilaremic individuals, also detects CFA in a proportion of amicrofilaremic individuals, suggesting that these

people have ultralow or single-sex (i.e., hidden) infections (Ottesen et al., 1997; Simonsen and Dunyo, 1999). In the present study, the ICT test was able to detect infection in 24.7% of parasitologically mf-negative individuals. This finding implies that the prevalence of the infection in most endemic areas is higher than previously estimated. The true prevalence of *W. bancrofti* infection in the current study was about twice that shown by the parasitologic methods. A similar observation has previously been made using the monoclonal antibody-based Og4C3 ELISA assay (Wamae et al., 1998).

In a study to assess the performance of ICT test as a diagnostic method for lymphatic filariasis in French Polynesia (Nguyen et al., 1999), the agreement between serum-based and whole-blood-based card tests was found to be high (100%). However, the study used stored sera for both the serum-based and whole-blood-based tests. In comparison with the Og4C3 ELISA, the sensitivity and specificity of the serum-based ICT test were 51.2% and 98.4%, respectively. As the whole-blood-based ICT test is a version designed for use with whole blood, it is important to compare the whole-blood-based ICT test (using whole blood) with the Og4C3 ELISA to empirically draw conclusions about their comparative sensitivities.

Fingerprick blood-based detection of mf has been shown to be more sensitive than a simple volumetric comparison of venous and fingerprick blood sample sizes would suggest, because of the concentration of mf in the capillary beds (Eberhard et al., 1988; Dickerson et al., 1989). In the present study, this observation was corroborated by the finding that the venous blood-based Knott's method gave lower prevalence and intensity of infection compared with the fingerprick blood-based counting chamber method. The finding implies that where mf counts are required to complement the qualitative ICT filariasis card test results, the less laborious counting chamber method, which is being extensively used in both East and West Africa (Estambale et al., 1994; Dzodzomenyo et al., 1999), is a better alternative. It may be argued that the commercially available counting chamber is too expensive (around \$40 [U.S.]), but the fact that it can be reused reduces the overall cost per test. Furthermore, homemade chambers are relatively easy to design and work just as well as the commercially available ones.

Most communities in filariasis-endemic regions are used to fingerprick blood collection for malaria diagnosis, and thus diagnosis of *W. bancrofti* infection using the fingerprick whole-blood version of the ICT card test may be more acceptable than venipuncture-based methods. Furthermore, the whole-blood version of the ICT filariasis card test will be a useful tool for diagnosis of bancroftian filariasis in remote rural areas where night blood sampling is not possible because of insecurity. Additionally, the card test is applicable to clinical settings where a qualitative result is required to rationalize treatment.

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