Comparison between the Skin Snip Test and Simple Dot Blot Assay as Potential Rapid Assessment Tools for Onchocerciasis in the Postcontrol Era in Ghana

G. E. Guzmán,1 K. Awadzi,2 N. Opoku,2 R. B. Narayanan,3 and H. O. Akuffo1*

Microbiology and Tumorbiology Center, Karolinska Institute and Swedish Institute for Infectious Disease Control, S-171 77 Stockholm, Sweden;1 Onchocerciasis Chemotherapy Research Centre, Hohoe Hospital, Hohoe, Ghana2; and Centre for Biotechnology, Anna University, Chennai 600 25, India3

Received 7 January 2002/Returned for modification 16 April 2002/Accepted 28 May 2002

Successful control of onchocerciasis through mass distribution of ivermectin needs to be coupled with reliable, sensitive, specific, yet affordable diagnostic methods to monitor and ensure the efficacy of such measures. The effort put into the development of diagnostic methods for onchocerciasis that can substitute for or work in combination with the present “gold standard,” the skin snip test, has resulted in the discovery of a number of immunogenic proteins with potential use as diagnostic tools in the postcontrol era. Most of these proteins have now been produced through recombinant DNA techniques. However, when costs are not a trivial issue, none of them have yet found their way into the areas where the disease still exists. In the present study, we have evaluated the performance of a simple dot blot assay which uses a mixture of native proteins designated PakF as a serious contender in the quest for a less invasive and more sensitive method to detect Onchocerca volvulus infection in areas with diverse endemicities. Our results indicate that the assay we propose is more sensitive than the skin snip test and shows high specificity, both characteristics required for a suitable tool for the monitoring of onchocerciasis in the postcontrol era.

Onchocerciasis (river blindness) occurs in 34 countries in Africa, Latin America, and the Arabian Peninsula. The vast majority of the estimated 18 million persons infected with the causative agent, the parasite Onchocerca volvulus, live in Africa (4). One of the goals of the World Health Organization is to eliminate onchocerciasis as a public health problem. The classical method to detect O. volvulus infection consists of examining small skin biopsy specimens (skin snips) by microscopy for the presence of O. volvulus microfilariae (mf). However, this very specific method is invasive. The process may be uncomfortable, which may result in poor compliance from individuals, especially in communities subjected to repeated tests (11). Much effort has been put into the development of less invasive serodiagnostic methods (5, 14, 19) to support the initiatives aimed at elimination of the disease. The program in West Africa, known as the Onchocerciasis Control Program (OCP), has been successful in meeting its goal of eliminating onchocerciasis as a public health problem in the 11 countries where the program has been active (1). As a result of the OCP’s success, the efforts of the program are now turning to surveillance, with the goal of rapidly detecting and controlling outbreaks of infection in the onchocerciasis-free zones (15). In the postivermectin era, however, there is still no diagnostic test available for large-scale use in onchocerciasis-free zones or areas where the disease is still endemic, although with very low mf density.

We have previously described a simple dot blot assay (DBA) using a native low-molecular-weight antigen fraction of female O. volvulus parasites, designated PakF (12), which is a useful diagnostic test in settings where the disease is nonendemic (13), and with its high specificity and sensitivity, it could have potential as a screening tool for onchocerciasis under certain circumstances. The DBA using this antigenic fraction (PakF-DBA) showed very high sensitivity and specificity when tested in Guatemala (9a), a country where onchocerciasis is endemic but where no other human filariasis has been reported (20). In this study, we describe the evaluation of the PakF-DBA for sera from individuals from localities with diverse endemicities in the Volta region of Ghana and compare the results with those obtained using the skin snip test. This region of Ghana has pockets of high and low transmission of onchocerciasis, and mass treatment has been practiced in some of these areas. Screening for onchocerciasis in these areas has always been done using the skin snip test; however, repeated ivermectin treatment results in decreased mf load, making the skin snip test less sensitive and reliable. We also evaluate the potential cross-reactivity of the assay with other filarial infections and show that sera from patients with circulating Wuchereria bancrofti do not cross-react in this assay. We discuss the potential use of the PakF-DBA as a less expensive and therefore affordable method for rapid screening and monitoring of areas where control measures have been established.

(G. Guzmán conducted this research in partial fulfillment of the requirements for his Ph.D. thesis at Karolinska Institute, Stockholm, Sweden, 2002.)

MATERIALS AND METHODS

Study population. The study was carried out in five localities of the district of Ho in the Volta region of Ghana with diverse endemicities (hyper- and hyp endemicity). Thirty individuals, who volunteered to participate, were selected from each locality, half of whom were within the age range of 5 to 17 years.
Informed consent was obtained from all the participants in the study or their parents or guardians. The localities were as follows: (i) Afapke (LOC1; male-to-female ratio, 0.9; child-to-adult ratio, 1), (ii) Kla (LOC2; male-to-female ratio, 0.9; child-to-adult ratio, 1), (iii) Aboe, Anorme, Asifiafe, and Luvudo (LOC3; male-to-female ratio, 2.3; child-to-adult ratio, 1), (iv) Honuta Gborgame (LOC4; male-to-female ratio, 0.8; child-to-adult ratio, 1), and Kpedze Anoe (LOC5; male-to-female ratio, 1.0; child-to-adult ratio, 1).

Study samples. Capillary blood was obtained by prickling the middle finger with a disposable hem lancet. Five circles of blood were blotted onto previously labeled 90-mm-diameter round, fast-drying filter paper (no. 597; Schleicher & Schuell, Dassel, Germany). When the blood spots were dry, filters were placed inside labeled plastic zip-lock bags and stored in the freezer compartment of a refrigerator (approximate temperature, −20°C). Blood collection was done according to the ethical and safety policies in Ghana. Pools of sera from mf− and nonexposed Guatemalan individ uals from a previous study were used as positive and negative controls, respectively. A panel of Indian sera from areas where *W. bancrofti* is endemic, including 9 microfilaricme patients (*W. bancrofti* mf+; age range, 10 to 40 years) and 16 patients with chronic infection but no circulating mf in the blood (chronic patients [CP]; age range, 20 to 50 years), were used as other filaria controls. In addition, 15 individuals living in the same area with no evidence of filariasis (endemic controls [EN]) were included. These sera were from an area where onchocerciasis is not reported.

Diagnosis of onchocerciasis. Parasitological examination for *O. volvulus* was done by four-site skin snips and microscopic examination for the presence of mf following 24-h incubation in saline at room temperature (approximately 20°C). The presence of mf of *O. volvulus* in any of the skin snips confirmed the diagnosis. Skilled technicians could distinguish *O. volvulus* from other filaria, such as *Wuchereria*, which is prevalent in the study area. The microfilarial density (MFD) was determined by dividing the average number of mf by the average weight of the skin snips in milligrams. Thus, an mf− diagnosis corresponded to an individual with an MFD of <0.

Antigen preparation (TSF). Intact noncalcified nodules obtained in a previous study were digested by the collagenase method of Schulz-Key et al. (16). Female worms were washed and frozen at −70°C in RPMI 1640 medium containing gentamicin (0.2 mg/ml). Tris-soluble antigen fractions (TSF) were prepared as previously described and stored at −20°C (12). The total protein concentration was estimated by the Bradford assay (2).

IEC. PakF fractions were prepared separately from TSF by ion-exchange chromatography (IEC) as described previously, except that neither a fast protein liquid chromatography system nor a peristaltic pump was used (12). We simplified the IEC preparation by using a handheld ion-exchange column as described elsewhere (unpublished data). One hundred and twelve micrograms of TSF (1 to 1.3 μg/μl) was diluted to 0.5 ml in equilibration buffer (50 mM Tris-HCl, pH 9.0, containing 0.05% Tween 20). This was passed manually through a 1-ml HiTrap-Q ionic column (Pharmacia, Uppsala, Sweden) at a flow rate of 0.5 ml/min using a 1-ml syringe as a reservoir. The sample was eluted with equilibration buffer at 0.5 ml/min, and the eluate was collected in eight 350-μl aliquots before the buffer was changed to equilibration buffer containing 0.5 M NaCl. Two final 350-μl aliquots were collected using this buffer. Each fraction was tested for antigenicity and polypeptide composition using a DBA (see below) and one-dimensional gel electrophoresis as previously described (9).

Serum elution from blood spots. Blood was extracted as previously described (8). Briefly, one disk, 6 mm in diameter, was cut out from a blood blot on each filter and extracted for 2 h in 2 ml of phosphate-buffered saline (PBS; pH 7.4) containing 0.05% Tween 20 (PBST). After slow vortexing, the disk was removed and the eluted serum was collected. In some instances, eluted sera were tested before and after heat treatment at 56°C for 30 min.

DBA. The DBA was performed as described previously with some modifications (12). Briefly, nitrocellulose strips, 4 mm wide and 3 cm long, were placed in mini-incubation trays (Bio-Rad Laboratories, Richmond, Calif.), and droplets (0.5 μl) of unconcentrated and undiluted PakF (approximately 5 ng) and TSF (64 ng; positive control) were adsorbed on the strips. The strips were allowed to dry at room temperature and were then blocked with 1% nonfat milk powder in PBST for 1 h under gentle agitation on a rocking shaker. The strips were incubated for 1 h with 1 ml of eluted serum. The strips were washed three times for 5 min each time with PBST and then incubated for 1 h with alkaline phosphatase-labeled goat anti-human immunoglobulin G (Calbiochem, La Jolla, Calif.) diluted 1:2,000 in PBST. The strips were washed three times as before with PBS and twice with PBST only before development of the immunoreactive spots with BCIP (5-bromo-4-chloro-3-indolylphosphate)-nitroblue tetrazolium (Sigma Chemical Co., St. Louis, Mo.). The color reaction was allowed to proceed for 10 min and then was stopped with several washes with distilled water. The strips were air dried in the dark. The DBA was performed blind, and thus the results from the skin snips were not made available to the person performing the assay until after all the analyses had been done. Control sera were tested first, diluted 1:4,000 in PBST.

Evaluation of the dot blots. Nitrocellulose strips were evaluated by visual examination and, for purposes of quantification, also by densitometry. Densitometric values were obtained as the integrated intensity of all the pixels in a spot excluding the background and were expressed as arbitrary units (Bio Image, Ann Arbor, Mich.). A visually positive sample was determined by identifying two spots of reactivity where PakF had been adsorbed, as well as the procedural control (TSF), are recognized by the serum sample. Negative samples do not show reactivity against PakF, although they may still react against some proteins present in the whole soluble extract, TSF. (C) Graphic representation of the densitometric data obtained by measuring the densities of the spots in all the individual DBA strips representing the five localities under study. A line at 0.1 U of integrated intensity indicates the cutoff value for visually negative samples.

![FIG. 1. (A) Reproducibility in the production of PakF. Lanes 1 to 4 show the electrophoretic patterns of PakF fractions obtained at different times, showing the mixture of at least five distinct bands that can be detected by silver staining. (B) Clear-cut difference between positive (+) and negative (−) samples in the PakF-DBA test. In a typical onchocerciasis-positive sample, the two spots where PakF had been adsorbed, as well as the procedural control (TSF), are recognized by the serum sample. Negative samples do not show reactivity against PakF, although they may still react against some proteins present in the whole soluble extract, TSF. (C) Graphic representation of the densitometric data obtained by measuring the densities of the spots in all the individual DBA strips representing the five localities under study. A line at 0.1 U of integrated intensity indicates the cutoff value for visually negative samples.](image)
RESULTS

Skin snip test results. Of the 150 individuals tested, 99 were skin snip positive and 51 were skin snip negative. A cluster of 40 negative individuals was found in the age range 5 to 20 years, and the remaining 11 were distributed between 21- and 60-year-olds (Table 1). No *Streptocerca* mf were detected in the skin snips of any of the participants in the study. The prevalence of individuals with mf skin snips varied from 43.3 to 76.7% in the five localities (Table 1). A closer evaluation of the <18-year-old age group shows that only one of the 15 individuals in LOC2 had a positive skin test, resulting in a prevalence of 6.7% (Table 3). In this age group, the highest prevalence was in locality LOC3.

DBA results. Figure 1A shows the reproducibilities in the production of PakF in different ages. Figures 1B shows the result of a positive DBA test. For a sample to be DBA positive, the two dots where PakF has been adsorbed need to show reactivity. TSF, included as a procedural control, may or not be reactive depending on the degree of cross-reactivity of the proteins in the whole soluble extract. In the case of onchoceriasis, infected individuals normally recognize TSF even if they do not recognize PakF, as they may react to other proteins present in the whole extract. In order to represent these results in a more graphic manner, densitometric values were generated for each sample, obtaining the mean of the integrated intensity of each pair of spots on each DBA strip. Using this system, we were able to assign a numeric value of intensity to spots which were visually weak or very weak. Those visually negative fell in the intensity range of 0 to 0.1, and those visually positive had an integrated intensity above 0.1. This allowed us to generate a graph of integrated intensities for the entire study population (Fig. 1C).

Figures 2 and 3 show the results obtained by DBA in all the areas under study. While the individual intensities of the spots varied, there was a clear-cut difference between positive and negative controls. In total, 125 samples were positive by DBA and 25 were negative, with a cluster of 22 DBA-negative samples within the age range 5 to 17 years. Individual analysis of each locality showed that the majority of positive individuals in the age range 5 to 17 years were inhabitants of the villages grouped as LOC3 and those from LOC5 (Table 3). The MFD in relation to age followed a distribution pattern very similar to that of the DBA versus age (not shown).

Comparison of DBA and skin snip results. Tables 2 and 3 show the comparisons of the DBA and the skin snip test results, using visual assessment of the strips, for the whole study population and those aged 5 to 17 years, respectively. Stratified by age (Table 1), the results clearly show that the DBA results closely followed the skin snip results in all age groups. However, the DBA consistently showed more positive individuals in all groups except the older age groups, with the biggest discrepancy between the DBA and skin snip test seen in the younger age groups.

A closer look at the 5-to-17-year-old age group in the individual localities (Table 3) showed a similar trend between the DBA and skin snip test, with LOC2 having the lowest DBA positivity but LOC5 showing DBA prevalence akin to that of LOC3.

The PakF-DBA detected more positive individuals than the skin snip test. Moreover, 98 out of the 99 skin snip-positive individuals were detected by DBA, for a sensitivity of 99% and a predictive value of 78%.

A positive correlation (Pcc = 0.899; P < 0.05) between the prevalences estimated by the skin snip test and PakF-DBA was found when the whole study population was analyzed, which did not vary appreciably when the 5-to-17-year-old population

---

TABLE 1. Comparison of PakF-DBA and skin snip test in diagnosis of onchocerciasis in different age ranges

| Age (yr) | Skin snip<sup>a</sup> | PakF-DBA<sup>b</sup> | PakF-DBA sensitivity (%)<sup>c</sup> |
|----------|----------------|----------------|----------------------------------|
|          | No. (%) POS | No. (%) NEG | No. (%) POS | No. (%) NEG | |
| 5–10     | 21 (14.0) | 35 (23.3) | 35 (23.3) | 21 (14.0) | 166 |
| 11–20    | 15 (10.3) | 5 (3.3) | 19 (12.7) | 1 (0.7) | 126 |
| 21–30    | 11 (7.3) | 1 (0.7) | 12 (8.0) | 0 (0.0) | 109 |
| 31–40    | 15 (10.3) | 4 (2.7) | 18 (12.0) | 1 (0.7) | 120 |
| 41–50    | 17 (11.3) | 5 (3.3) | 20 (13.3) | 2 (1.5) | 118 |
| 51–60    | 12 (8.0) | 1 (0.7) | 13 (8.7) | 0 (0.0) | 108 |
| 61–70    | 6 (4.0) | 0 (0.0) | 6 (4.0) | 0 (0.0) | 100 |
| 71–80    | 2 (1.3) | 0 (0.0) | 2 (1.3) | 0 (0.0) | 100 |
| Total    | 99 (66.0) | 51 (34.0) | 125 (83.3) | 25 (16.7) | |

<sup>a</sup> POS, MFD = 0 mf/mg of skin; NEG, MFD = 0 mf/mg of skin.
<sup>b</sup> POS, visually positive; NEG, visually negative.
<sup>c</sup> Sensitivity of DBA in individual age groups is defined in comparison with the skin snip results.

---

TABLE 2. Performance of PakF-DBA compared to skin snip test for diagnosis of onchocerciasis in the age range 5 to 17 years

| Locality | Skin snip<sup>a</sup> | PakF-DBA<sup>b</sup> |
|----------|----------------|----------------|
|          | No. (%) POS | No. (%) NEG | No. (%) POS | No. (%) NEG |
| LOC1     | 15 5 (33.3) | 10 (66.7) | 5 (33.3) |
| LOC2     | 15 1 (6.7) | 14 (93.3) | 6 (40.0) |
| LOC3     | 15 13 (86.7) | 2 (13.3) | 13 (86.7) |
| LOC4     | 15 8 (53.3) | 7 (46.7) | 10 (66.7) |
| LOC5     | 15 6 (40.0) | 9 (60.0) | 14 (93.3) |
| Total    | 75 33 (44.0)<sup>c</sup> | 42 (56.0)<sup>c</sup> | 53 (70.7)<sup>c</sup> |

<sup>a</sup> POS, MFD = 0 mf/mg of skin; NEG, MFD = 0 mf/mg of skin.
<sup>b</sup> POS, visually positive; NEG, visually negative.
<sup>c</sup> Mean value of all localities.

---
was analyzed separately ($P_{cc} = 0.893; P > 0.05$). There was no significant correlation ($r = 0.102; P > 0.05$) between the prevalences estimated by the two methods among those older than 18 years.

**Cross-reactivity of PakF-DBA with other filarial sera.** The specificity of the PakF-DBA was assessed using sera from Indian individuals with bancroftian infection with apparently no exposure to *O. volvulus*. None of the *W. bancrofti* mf$^+$ sera or the control sera were positive by DBA. However, two CP and two EN sera reacted positively on the DBA (Fig. 4). Pooled Guatemalan mf$^+$ and nonexposed sera, used as positive and negative controls, were positive and negative, respectively, when tested at the same dilution. In total, 4 out of 40 Indian sera were positive by DBA, resulting in a 90% specificity of the assay.

**DISCUSSION**

Since the advent of successful control of onchocerciasis through measures including mass distribution of ivermectin, the need for developing surveillance programs to detect recrudescence of infection in the OCP area, as well as safe, inexpensive, and rapid diagnostic tests for *O. volvulus* infection, has been highlighted. In the present study, we have evaluated the performance of a simple DBA (PakF-DBA) as a serious contender in the quest for a less invasive and more sensitive method to detect *O. volvulus* infection in areas with diverse endemicities. With the PakF-DBA, we were able to detect a trend similar to that of the skin snip test, but the PakF-DBA detected a higher proportion of infected individuals than the skin snip test. These included 98 out of 99 detected by the more invasive gold standard test. This could indicate that the sensitivity of the DBA described here is higher than that achieved with the skin snip test.

Although we have used analytical densitometry to generate comprehensive graphs, we have shown that the results obtained by visual examination of the DBA strips correspond well with densitometric data (Guzmán et al., submitted). The clear-cut difference between positive and negative samples allows for visual assessment instead of the use of a sophisticated apparatus such as a densitometer. This is of particular interest in areas where the use of delicate or sophisticated readout devices is not practical. Therefore, the visual results of the DBA strips were enough to establish a correlation between the prevalences estimated by the skin snip test and those estimated by DBA in all age groups. The fact that the DBA results were comparable to those obtained by skin snip tests indicates the possibility that the overall estimates of prevalence generated by DBA from a sentinel population could replace those generated by skin snips. The major difference in the number of positive individuals detected by DBA and skin snip test was observed in the groups aged 5 to 17 years (Table 2). When analyzed separately, a positive and significant correlation between the prevalences estimated with the PakF-DBA and the skin snip test was found for the population aged 5 to 17 years. However, no such correlation was observed in the group of adults studied (>17 years old). Due to the long life span of the adult worms, the continuous exposure in areas with ongoing transmission of onchocerciasis, and the unknown duration of circulation of antibodies against the proteins contained in
PakF, it is not likely that an antibody-based assay like the PakF-DBA would show a better performance than the skin snip test when an age group of $>20$ years is selected as a sentinel population to assess the efficacy of control measures. Our results are in accordance with those of other studies that have shown that the most useful indicator group for detecting recrudescence, as well as for determining the intensity of infection in a community, is the population 5 to 15 years of age.

FIG. 3. DBA results from LOC2, LOC3, LOC4, and LOC5. Visual assessment of the DBA strips was performed as indicated in the legend to Fig. 2.

FIG. 4. Specificity of PakF-DBA. The graphic representation of densitometric data shows that the PakF-DBA is highly specific when sera from individuals with other filarial infections, other than onchocerciasis, are analyzed. Two $W. bancrofti$ CP and two EN were positive by PakF-DBA, whereas none of the microfilaremic (MF) individuals reacted to PakF. Pools of Guatemalan $O. volvulus$-infected (GUmf$^+$) and nonexposed (N-GU) samples were used as positive and negative controls, respectively. Visual assessment: samples classed as negative had an integrated intensity of $<0.1$ U, whereas those classed as positive had an integrated intensity of $>0.1$ U.
The PakF-DBA may be positive in more specimens due to a number of reasons, including higher sensitivity, lower specificity, or the capacity to detect even early (prepatent) infection. The possibility of detecting prepatent disease can only be evaluated in long-term longitudinal studies. Our results suggest that the PakF-DBA has a higher sensitivity coupled with high specificity.

There is an emphasis in the World Health Organization strategy on the need for surveillance methods to be highly specific even at the cost of low sensitivity. The problem of specificity is that of the quality of the gold standard. If the gold standard has low sensitivity, then assays with higher sensitivities can be viewed as having low specificities. This is the problem with the highly specific but low-sensitivity skin snip test. However, we have previously established the specificity of the PakF-DBA assay to be 100% using a panel of sera from non-infected individuals from areas where no other filaria is present (Guzmán et al., submitted). In the later studies, we used sera from individuals infected with other coendemic parasites in the area, mainly Ascariis lumbricoides, in order to rule out any cross-reactivity due to the carbohydrate-containing antigens widely shared among nematodes, primarily phosphocholine-containing antigens (10). In addition, we ruled out cross-reactivity with a series of sera from filaria-infected patients. In the present study, we have also approached this question by extending the pool of test samples with well-characterized sera from individuals with other filarial infections from areas where onchocerciasis is not endemic. The sensitivity of the PakF-DBA using these sera was 90%. Of importance, however, is the fact that those positive in the PakF-DBA were not the individuals with circulating bancroftian mf, for whom nine of nine sera were negative by the PakF-DBA test. Combined analysis using the present data and the additional panels of sera from areas where other filaria, but not O. volvulus, are endemic resulted in 96% specificity (Guzmán et al., submitted).

These results also suggest that the PakF-DBA could be useful in screening children and adolescents born after control measures have been established, since they form a desirable sentinel population to detect recrudescence of infection in controlled areas (11).

In the areas of endemicity, the need for assays that provide rapid results is advocated. The time needed to obtain qualitative results from the PakF-DBA was comparable to that for the rapid version of the skin snip test, i.e., 3 h. Thus, although a quantification of the level of infection is beyond the capabilities of the DBA, in terms of a rapid assessment for surveillance purposes, it represents an affordable alternative that offers more sensitivity than the skin snip test. Moreover, the test can be performed using only a few drops of finger prick blood, and this represents an advantage in terms of a higher compliance from the individuals in areas under constant screening to evaluate the impact of control measures.

Several promising diagnostic methods, based on detection of specific antibodies, parasite DNA, or parasite antigens in clinical specimens, have been developed for onchocerciasis in recent years (6, 7, 14, 21). However, none of these have been implemented under field conditions in the areas of endemicity. Antibody tests using recombinant antigens have been based on standard, indirect enzyme-linked immunosorbent assays, which can be performed in a simple laboratory. The antigen and DNA detection methods are performed over 2 or 3 days, and they require more, in terms of laboratory infrastructure, equipment, and expensive reagents, than the enzyme-linked immunosorbent assay (18). In either case, the costs involved in both the acquisition of instrumentation and the transfer of the technology are not trivial, thus making the development of less expensive, stable, and reliable methods a necessity in low-income countries where onchocerciasis is being controlled or still exists. Simple yet reliable approaches like the use of a less expensive diagnostic technique that could be implemented under field conditions are of particular importance, especially when the successor to OCP, the African Program of Onchocerciasis Control, is facing funding difficulties which could threaten its future success (17).

The PakF-DBA described here, unlike antigen and DNA detection methods, does not require pretreatment of samples or expensive supplies and equipment (e.g., PCR). We have evaluated the PakF-DBA in heat-inactivated eluted sera (56°C; 30 min) and found no difference in intensity between heated and unheated sera (data not shown). In addition, we have observed that PakF is highly stable at working temperatures normally found in the areas of endemicity, and it does not require the use of anti-immunoglobulin G4 conjugates to reduce the background, since the molecules comprising PakF do not seem to be recognized by such an isotype (Guzmán et al., submitted). A possible disadvantage, compared to antigen or DNA detection methods, is that the PakF-DBA does not distinguish between past and present infections, but this is a problem of all antibody-based assays. Nevertheless, the PakF-DBA may still be a suitable tool for following changes in transmission when used in younger age groups. Furthermore, antibody testing still holds great promise as a means for monitoring changes in the transmission of O. volvulus after mass treatment of populations (18).

Criticsms of the use of native human-derived material in the PakF-DBA have previously been made in terms of reproducibility of material and safety. We have made many preparations of PakF, and the reproducibility in performance in the DBA has been high. A reliable, sensitive, and specific recombinant protein would be valuable, but in its absence, native proteins still have a role. Where the question of possible exposure to contaminant viruses is concerned, PakF, which is a protein fraction stable at high temperatures, may be heat treated before use (Guzmán et al., submitted). In addition, the PakF-DBA can be used with heat-inactivated sera.

Concern about the availability of parasite material to prepare PakF is justified. However, in areas where no control measures have been established, the relative abundance of nodules from which the worms are freed makes it possible to obtain large amounts of PakF to be used in rapid screening of sentinel populations. In our hands, the amount of TSF obtained from a batch of one to three worms is enough to run a HITrap-Q column up to 10 times. Each purification round produces 350 μl of PakF that can be applied to nitrocellulose strips without further treatment. The yield after 10 purification rounds (3.5 ml) allows the analysis of at least 3,000 samples, using the format of duplicate PakF spots per strip. Even if the format is changed to use 1 μl per spot, the number of samples that could be tested is large enough, considering that the ma-
terial comes from only one to three worms. In the areas of endemicity, where nodulectomy is a common practice not only for control but also for aesthetic purposes, the availability of worms for PakF production would not be an insurmountable difficulty.

We and others have shown variations in the protein compositions of *O. volvulus* parasites from different geographic areas (9). However, we have prepared PakF from worms isolated in both Ghana and Guatemala, used in crossed analysis with sera from the two countries, and obtained similar results regardless of the origin of the parasite material (Guzmán et al., submitted). Therefore, geographical variations would not be of concern in screening a particular area with parasite material obtained from worms from another region.

In conclusion, the present study provides evidence that it is still possible to implement simple techniques for the monitoring of onchocerciasis which offer high sensitivity and specificity while being affordable in financially challenged areas. Where costs are an issue, and in the absence of more elaborate diagnostic tests in the areas of endemicity, simple tests like the PakF-DBA could still deliver reliable information that can be used to monitor changes in the transmission of onchocerciasis.

ACKNOWLEDGMENTS

G. Guzmán has been supported by Sida/SAREC (the Swedish International Development Agency) through the Karolinska Institute Research and Training Program (KIRT).

We thank the staff at the Onchocerciasis Chemotherapy Research Center (OCRC), Hohoe Hospital, Hohoe, Ghana, for their contributions and technical assistance with patients and skin snip handling.

REFERENCES

1. Boatin, B. A., L. Toé, E. S. Alley, N. Dembéle, N. Weiss, and K. Y. Dadzie. 1998. Diagnostics in onchocerciasis: future challenges. Ann. Trop. Med. Parasitol. 92:541–545.

2. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

3. Bradley, J. E., B. M. Atogho, L. Elson, G. R. Stewart, and M. Bussinesq. 1998. A cocktail of recombinant *Onchoerca volvulus* antigens for serologic diagnosis with the potential to predict the endemicity of onchocerciasis infection. Am. J. Trop. Med. Hyg. 59:877–882.

4. Burham, G. 1998. Onchocerciasis. Lancet 351:1341–1346.

5. Chandrashekar, R., K. Massod, R. M. Alvarez, A. F. Ogurinrnade, R. Lujan, F. O. J. Richards, and G. J. Weil. 1991. Molecular cloning and characterization of recombinant parasite antigens for immunodiagnosis of onchocerciasis. J. Clin. Investig. 88:1464–1471.

6. Chandrashekar, R., A. F. Ogurinrnade, R. M. Alvarez, O. O. Kale, and G. J. Weil. 1990. Circulating immune complex-associated parasite antigens in human onchocerciasis. J. Infect. Dis. 162:1159–1164.

7. Chandrashekar, R., A. F. Ogurinrnade, and G. J. Weil. 1996. Use of recombinant antigens for diagnosis and surveillance of human onchocerciasis. Trop. Med. Int. Health 1:575–580.

8. Evengeard, B., N. Gagi, and E. Linder. 1988. A filter-paper technique for the detection of IgG and IgM class schistosome-specific antibodies in an endemic area. Ann. Trop. Med. Parasitol. 82:307–309.

9. Guzmán, G. E., H. O. Akuffo, C. Lavebratt, and R. Luján. 1997. Immune response to *Onchocerca volvulus*: IgG1 antibody responses differ in onchocerciasis patients from Ghana and Guatemala. Acta Trop. 63:15–31.

10. Guzmán, G. E., C. Lavebratt, R. Luján, and H. O. Akuffo. Diagnosis of onchocerciasis using highly specific native proteins. Scand. J. Infect. Dis., in press.

11. Lal, R. B., R. R. Dhanwan, J. T. Tarrand, E. M. Ayoub, and E. A. Ottesen. 1991. Lack of IgG4 antibody response to carbohydrate antigens in patients with lymphatic filariasis. Immunology 74:333–337.

12. Laurent, T., A. G. Adjami, B. A. Boatin, C. Back, E. S. Alley, N. Dembéle, P. G. Brika, E. Pearlman, and T. Unnasch. 2000. Topical application of diethylcarbamazine to detect onchocerciasis recrudescence in West Africa. Trans. R. Soc. Trop. Med. Hyg. 94:519–525.

13. Lavebratt, C., G. Dalhammar, N. A. Adamafio, U. Nkänen-Dejerud, K. Mångarini, K. Ingemarsson, N. Opoku, and H. O. Akuffo. 1994. A simple dot blot assay adaptable for field use in the diagnosis of onchocerciasis: preparation of an adult worm antigen fraction which enhances sensitivity and specificity. Trans. R. Soc. Trop. Med. Hyg. 88:303–306.

14. Lavebratt, C., P. H. Ljungström, G. E. Guzmán, C. Thors, T. Eriksson, and H. O. Akuffo. 1997. Evaluation of serological assays for diagnosis of onchocerciasis. Scand. J. Infect. Dis. 29:65–70.

15. Lobos, E., N. Weis, M. Karam, H. R. Taylor, E. A. Ottesen, and T. B. Nutman. 1991. An immunogenic *Onchocerca volvulus* antigen: a specific and sensitive end point marker of infection. Lancet 338:1603–1605.

16. Molynieux, D. H., and J. B. Davies. 1997. Onchocerciasis control: moving towards the millennium. Parasitol. Today 13:418–424.

17. Schulz-Key, H., E. J. Albiz, and D. W. Büttner. 1977. Isolation of living adult *Onchocerca volvulus* from nodules. Trop. Med. Parasitol. 28:428–430.

18. Stevenson, P. 1999. ACCRA Vision is failing for river-blindness control in West Africa. J. Clin. Investig. 104:1460–1466.

19. Vincent, J. A., S. Lustigman, S. Zhang, and G. J. Weil. 2001. Evaluation of newer tests for the diagnosis of onchocerciasis. Ann. Trop. Med. Parasitol. 95:253–258.

20. Weil, G. J., C. Steel, F. Lefit, G. Mearns, E. Lobos, and T. B. Nutman. 2000. A rapid-format antibody card test for diagnosis of onchocerciasis. J. Infect. Dis. 182:1796–1799.

21. Weil, G. J., C. Steel, F. Lefit, G. Mearns, E. Lobos, and T. B. Nutman. 2000. A rapid-format antibody card test for diagnosis of onchocerciasis. J. Infect. Dis. 182:1796–1799.

22. Zea-Flores, G. 1985. Epidemiología de la oncocercosis en Guatemala, p. 76–80. In L. Yarzabal, C. Botto, and R. Allan (ed.), La oncocercosis en América Latina, vol. 3. Editorial La Galera de Artes Gráficas S. R. L., Caracas, Venezuela.

23. Zimmerman, P. A., R. H. Guderian, E. Arajuo, L. Elson, P. Phadke, J. Kuboffic, and T. B. Nutman. 1994. Polymerase chain reaction-based diagnosis of *Onchocerca volvulus* infection: improved detection of patients with onchocerciasis. J. Infect. Dis. 169:686–689.