RESEARCH NOTE

COMPARISON OF TWO IgG4 ASSAY FORMATS (ELISA AND RAPID DIPSTICK TEST) FOR DETECTION OF BRUGIAN FILARIASIS

Rahmah Noordin[1], Ranganatha K Shenoy[2] and Rohana A Rahman[1]

[1]Department of Medical Microbiology/Parasitology and Center for Medical Innovations and Technology Development, Health Campus, Universiti Sains Malaysia, Kelantan, Malaysia; [2]Filariasis Chemotherapy Unit, TD Medical College Hospital, Allepey, Kerala, India

Abstract. *Brugia malayi* infection is endemic in several Asian countries. Filaria-specific IgG4 antibody detection based on *BmR1* recombinant antigen has been shown to be sensitive and specific for the diagnosis of brugian filariasis. Two formats of the test has been reported *ie* indirect ELISA (BE) and rapid dipstick test (BR). Since different test formats use different amounts of sample and reagents which may affect its sensitivity and specificity, this study was performed to compare these two test formats in the detection of *B. malayi*. A total of 264 blinded serum samples from India and Malaysia were employed. Group 1 comprised 164 samples from actively infected individuals and group 2 comprised 100 samples from filaria non-endemic areas. Sensitivity was 96.3% (158/164) and 90.8% (149/164) for rapid test and ELISA respectively; chi-square p=0.00. Both test formats demonstrated 100% specificity. Therefore the rapid test format was equally specific but more sensitive than the ELISA format. The ELISA format would be able to demonstrate decline in IgG4 titer post-treatment while the rapid test would be very useful for screening and diagnosis in the field.

Brugia infections accounts for ~ 10% of the 120 million people infected with lymphatic filariasis (WHO, 1997). This species is endemic in Indonesia, India, Malaysia, Thailand, Vietnam, Philippines and South China. The routine detection method is by microscopy of night blood. However, this technique is insensitive and leads to many undetected cases (Turner *et al*, 1992). A diagnostic method with high sensitivity and that does not require night blood sampling is urgently needed to assist in the ongoing WHO-initiated global program for the elimination of lymphatic filariasis.

Two serological assay formats *ie* rapid dipstick test (Brugia Rapid, BR) and ELISA (Brugia-Elisa, BE), both of which utilized the same recombinant antigen (*BmR1*), have been previously reported to demonstrate good sensitivity and specificity for the detection of brugian filariasis (Rahmah *et al*, 2001a,b). Both tests used the same principle of binding the recombinant antigen to a filarial-specific human IgG4 antibody in the patient sample, followed by binding of the complex to mouse anti-human IgG4 monoclonal antibody. The two test formats differ in the amounts of sample and reagents and in the methods of detection; these differences can affect the sensitivity and specificity of the test. This study was conducted to compare the sensitivity and specificity of these two test formats in the detection of *B. malayi* infection.

A total of 264 blinded serum samples were used in this study. Group 1 was comprised of 164 samples from actively infected individuals from brugian filariasis endemic areas in Kerala, India and group 2 was comprised of 100 samples from filarial non-endemic areas in Malaysia. Actively

Correspondence: Rahmah Noordin, Department of Medical Microbiology/Parasitology and Center for Medical Innovations and Technology Development, Health Campus, Universiti Sains Malaysia, 1615 Kubang Kerian, Kelantan, Malaysia.
Tel: 609-766 3370; Fax: 609-765 3370
E-mail: rahmah@kb.usm.my

infected people are defined as those who are microfilaremic or amicrofilaremics with adult worms in the lymphatics. Out of the 164 individuals, 156 had circulating microfilaria (mf) and 8 were amicrofilaremic. The latter comprised of treated individuals who were microfilaremic in samples shortly before the commencement of treatment. Three individuals were treated with one dose of 6mg/kg diethylcarbamazine (DEC) a day before sampling and 5 individuals received treatment 2 weeks before sampling; the treatment of the latter comprised of either one dose of invermectin (200 μg/kg) + DEC (6 mg/kg) or ivermectin + albendazole (400 mg) or DEC + albendazole. Since these individuals were treated shortly prior to sampling and did not receive a complete course of the drug(s), it would be expected that some of the adult worms would still be alive, thus they were considered to be actively infected at blood sampling time. The 100 sera from the group 2 samples came from non-filarial endemic areas ; they comprised 20 with soil-transmitted helminth infections, 10 with other helminthic infections, 20 with protozoan infections and 50 healthy people. All sera samples were from sera bank of each institution and had been previously collected according to the requirements of the ethics committee of each institution.

Table 1
Samples with low IgG4 responsiveness as indicated by negative serology results either with BR or BE or both.

No.	Code	No. mf/ml	BR	BE
1	15d	12	+	-
2	110b	516	+	-
3	110d	300	+	-
4	114	224	-	-
5	127a	403	-	+
6	127b	76	-	-
7	127c	36	-	-
8	127d	4	-	-
9	141b	16	+	-
10	145a	1,981	+	-
11	145b	640	+	-
12	146a	531	+	-
13	146c	0	-	-
14	147c	72	+	-
15	148a	146	+	-
16	149c	4	+	-

BR : Brugia Rapid; BE : Brugia Elisa
For all samples, BR and BE were performed at least twice to confirm the results. For BE, the tests were also repeated with varying amounts of antigen and serum concentrations to ensure the negative results were not due to a prozone effect .

The procedures employed for performing BR and BE were as previously reported (Rahmah *et al*, 2001a,b). For the BR test, the dipstick previously lined with *BmR1* recombinant antigen and control antibody (goat anti-mouse IgG) is placed in a microwell containing equal volumes of serum and buffer. When the dipstick was almost fully wetted, the sample pad was cut and the dipstick was placed in a reconstituted conjugate well (monoclonal anti-human IgG4 conjugated to colloidal gold). The results were read within 15 minutes. The appearance of two lines indicated a positive result while the appearance of one line indicated a negative result. For BE, a patient sample is added to the recombinant antigen-coated microtiter plate wells and incubated for 2 hours at 37°C. After a washing step, peroxidase conjugated monoclonal anti-human IgG4 is added and incubated for 30 minutes. Another washing step follows before ABTS substrate is added and the results read at 405 nm with a spectrophotometer.

When compared to microscopy, the group 1 samples showed that the sensitivity of BR was 96.3% (158/164) and the sensitivity of BE was 90.8% (149/164); this difference was found to be significant by chi-square analysis (p=0.00). Table 1 shows samples with negative results by the two tests. The percentage of individuals with IgG4 low responsiveness by BR was 3.7% (6/164), by BE was 9.2% (15/164), by either test was 9.8% (16/164) and by both tests was 3.0% (5/164). From the group 2 samples, both the BR and BE were negative thus demonstrating a 100% specificity and concordance in results between the two tests.

Previous multicenter laboratory evaluations showed that BE demonstrated sensitivity and specificity of 96-100% (Rahmah *et al*, 2001a); BR demonstrated sensitivities and specificities of 97% and 99% (Rahmah *et al*, 2001b); and 93% and 100% (Rahmah *et al*, 2003, in press) respectively. The false positive results in these previous studies and

in the present study can be attributed to low IgG4 responsivenesss in these individuals. This phenomenon was previously reported whereby ~ 10% of microfilaremic individuals in a bancroftian filariasis endemic areas were found to be negative in an IgG4 assay (Marley *et al*, 1995). In addition, patients with concurrent immunocompromising diseases may also show false negative results. The latter is exemplified by a case at Universiti Sains Malaysia hospital of a patient on chemotherapy who had non-Hodgkin's lymphoma and active brugian filariasis (microfilaremic). Filariasis serology using the Brugia Rapid dipstick was positive (albeit weakly positive); however Brugia-Elisa gave negative results (optical density of 0.07; cut-off OD is 0.300).

Thus the sensitivity of an assay will affect the percentage of low IgG4 responders reported in a population. We can expect the kind of antigen used in an IgG4 assay will affect the percentage of low-responders in a study. Thus, for the purpose of patient diagnosis, it is best to perform both microscopy and the IgG4 test to attain maximal sensitivity. However, due to the good sensitivity and high specificity of the IgG4 test, it may be used as the sole method for brugian filariasis screening.

It can be observed from Table 1 that there appeared to be no relationship between mf levels and low IgG4 responsiveness, since samples with negative serology (either BR- or BE-) had varied levels of microfilaremia *ie* five persons with low mf levels (less than 20 mf/ml), three with intermediate mf levels (<100 mf/ml) and seven with high mf levels. This is in agreement with the results of a previous study which showed no correlation between mf levels and ELISA results (Rahmah *et al*, 2001a).

Since Brugia ELISA provides quantitative results, it would be useful as a research tool and to demonstrate the decline in the filarial-specific IgG4 titer in post-treatment follow-ups. The lower sensitivity of ELISA will show clearance of the infection faster than by the rapid test. Brugia-Elisa could be applicable in situations where filariasis screening is performed in a designated laboratory by trained personnel, such as in the screening of foreign workers from endemic countries. ELISA can then be performed on many samples in one run, thus allowing for cost savings.

This study shows that Brugia Rapid is equally specific but more sensitive than Brugia-Elisa in detecting brugian filariasis. The dipstick format is being commercialized by Malaysian BioDiagnostics Research Sdn Bhd (MBDr). A second generation of the rapid test in a simpler cassette format is being developed. Due to its field-applicability, Brugia Rapid would be useful in filariasis screening in endemic areas, and for patient diagnosis, especially in remote areas.

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