Detection of day blood filarial antigens by Og4C3 ELISA test using filter paper samples

S. L. HÔTI, A. ELANGO, K. RADIJAME, J. YUVARAJ, S. P. PANI

ABSTRACT

Background. The launching of the global filariasis elimination programme has necessitated the use of highly sensitive and specific diagnostic tests. The Og4C3 monoclonal antibody-based ELISA test has been found to be highly specific and sensitive for the diagnosis of filariasis using night blood samples. However, it requires a serum sample which poses problems of transport and storage. Collection of blood samples on filter paper will greatly circumvent these problems. Therefore, we evaluated the utility of the Og4C3 assay on filter paper samples collected during daytime.

Methods. Blood samples were collected from 63 microfilariae (mf) carriers during different time periods in a day on filter paper discs as well as venous blood for sera. The mf carriers and chronic (hydrocele n = 20; lymphoedema n = 120) and acute filariasis (adenolymphangitis n = 39) patients were from the endemic areas and the non-endemic normals were from Uthangamandalam district of Tamil Nadu, India. The filarial antigens in the samples were determined using the Og4C3 filarial antigen assay as per the manufacturer's instructions (JCU TropBio, Australia). The sensitivity of the assay on sera and filter paper
samples collected during night and also on filter paper samples collected during different time intervals of the day were compared with those of the membrane filtration technique, which was used as a gold standard.

**Results.** The geometric mean titre of the sera samples collected during night was 11 units/ml for non-endemic normals and 601.2 units/ml for mf carriers. The specificity of the assay on sera samples collected during night was 100% and the sensitivity 96.8% and the positive and negative values were 100% and 95.2%, respectively. The antigen positivity of the filter paper samples collected during morning hours was 93.3% while it was 76.6% and 86.7% for afternoon and evening hours. A significant association was observed between antigenaemia levels and mf density in the blood samples collected during the night.

**Conclusion.** The samples collected on filter paper during the day can be used as an alternative to sera samples for detection of filarial antigens employing Og4C3 ELISA. Also, samples collected during morning hours yield a higher positivity. The assay when applied to serum samples will be useful especially when quantitative results are required.


**INTRODUCTION**

Attempts to develop immunodiagnostic tools for lymphatic filariasis in blood samples have resulted in at least 2 highly specific and sensitive tests, which are currently available commercially. The ICT™ filariasis card test, based on a monoclonal antibody AD12, is simple and requires no equipment, but is not quantitative. The TropBio™ *Wuchereria bancrofti* ELISA kit, based on another monoclonal antibody Og4C3, is a sandwich enzyme-linked immunosorbent assay (ELISA) which can generate quantitative information. Both these tests detect the circulating filarial antigens (CFA) in sera samples with a high specificity and sensitivity. Since the TropBio test can generate quantitative information, it can be used to investigate the dynamics of infection and development of disease.

The TropBio test was originally standardized for antigen detection in sera samples. However, if blood samples collected on filter paper discs could be used for the assay, it would facilitate easy transportation and storage and make it more useful for field studies. Lalitha et al. and Itoh et al. used filter paper samples for the assay and found that these could be used with equal efficiency. Simonsen and Dunyo compared the efficiency of three tests in detecting circulating filarial antigens, viz., (i) ICT card test for serum samples, (ii) the Og4C3 monoclonal based ELISA (TropBio) test for sera samples, and (iii) the Og4C3 monoclonal based ELISA (TropBio) test for filter paper samples. They found that all the three tests were promising diagnostic tools. However, in these studies only night blood samples (both for sera and filter papers) were used, the community acceptance of which is known to be poor. Therefore, we (i) evaluated the sensitivity and specificity of Og4C3 assay using sera/filter paper samples collected both during day and night, (ii) investigated the variations in the sensitivity of the assay due to the collection of samples during different periods of the day, and (iii) calculated the antigen positivity rate and levels in different groups of patients with filariasis.

**PATIENTS AND METHODS**

**Selection of cases**

The study included individuals from rural areas of Thiruvannamalai and Villupuram districts of Tamil Nadu, an endemic area for bancroftian filariasis. The following 5 groups of individuals were selected:

1. asymptomatic and microfilaraemic (endemic normals; *n*=24),
2. asymptomatic but microfilaraemic with a microfilarial (mf) density ranging from 1 to 250/60 cmmm and 1 to 6500/ml (*n*=63),
3. adenolymphangitis cases (*n*=39),
4. lymphoedema cases (*n*=120),
5. hydrocele cases (*n*=20).

Non-endemic normal sera (*n*=40) were collected from tribal residents in the hilly areas of Uthagamandalam district of Tamil Nadu, India. Except mf carriers all other cases were found to be negative for mf.

**COLLECTION OF BLOOD SAMPLES**

Venous blood samples (5 ml) were collected from all the above groups during the night (between 2000 and 2200 hours). One ml of blood was transferred to a heparinized tube for subsequent membrane filtration assay for mf count and the rest (4 ml) was used for separation of serum. The sera separated were stored at –20 °C until the assays were carried out. The heparinized venous blood samples (1 ml) were processed by membrane filtration, and stained with JSB stain using standard procedure. The stained membranes were examined for mf and the number of mf were counted and recorded.

**Collection of blood samples on filter paper discs**

Blood samples were collected from 30 of the 63 mf carriers on a filter paper during 4 different periods of the day: 0600–0800 hours, 1000–1200 hours, 1400–1600 hours and 2000–2200 hours. The selection of these parasite carriers was based on their availability and willingness to participate in the study. Twenty microlitre of blood, equivalent to 10 μl serum, collected by the finger prick method from each of these individuals was absorbed on to Whatman No.3 filter paper. The blood spots were air-dried and stored in polyethylene bags at –20 °C until further use. For comparison, parallel venous blood samples were also collected during 1000–1200 hours. Thus, for these 30 parasite carriers, sera samples were available for two time periods: 1000–1200 hours and 2000–2200 hours. Due to logistic reasons, it was not possible to collect sera samples at all the four periods of the day. The filter paper samples and the sera separated from the blood samples were stored at –20 °C until further use.

**Elution of filter paper spots**

The blood spots from filter papers were cut out and placed in microcentrifuge tubes containing 200 μl of phosphate buffered saline (PBS) for eluting the antigens. The dilution of the serum sample on a filter spot thus works out approximately to 1:20. The samples were eluted at 4 °C overnight.

**Assay for circulating antigen**

The circulating antigens of *W. bancrofti* in the sera and the filter paper eluate were quantified using the Og4C3 ELISA kit according to the instructions of the manufacturer (JCU Tropical Biotechnology Private Limited, Queensland, Australia). Fifty microlitre of either serum or filter paper eluate were used for the assay and each sample was tested in duplicate. The optical density (OD) was read at 414 nm. The OD values of 7 standard antigen controls provided in the kit were used to construct the standard curve. The antigen titre was determined for each individual using the mean OD values and the standard curve. Samples with an OD value of
The percentage positivity and the geometric mean titre of antigenaemia for each study group was calculated from the titre values. Chi-square test was used to compare the proportion of positivity between groups. Regression analysis was used to determine the association between prevalence of antigenaemia (titre) and mf count in the 63 parasite carriers.

RESULTS

The geometric mean titre (GMT) of antigenaemia in the sera samples collected during the night (2000–2200 hours), determined using the Og4C3 antigen detection test, was 11 units/ml for non-endemic normal individuals while that of the mf carriers was 601.2 units (Table I). This indicates that the mf carriers had very high antigen levels in their blood while non-endemic normal individuals had negligible level of antigens (p<0.05). Therefore, the test could clearly differentiate between infected and non-infected individuals. Of the 63 subjects detected as microfilaraemic by the membrane filtration technique, 61 were positive CFA. None of the 40 non-endemic normal individuals were positive for CFA. The specificity of the test therefore was 100% and the sensitivity 96.8%. The positive and negative predictive values for the test were 100% and 95.2%, respectively.

The test also detected CFA in 37.5% of the asymptomatic microfilaraemic endemic individuals (endemic normal), 50% of the hydrocele cases and 37.5% of the lymphoedema cases (Table I). However, the GMT for the lymphoedema and hydrocele cases was significantly lower when compared to that for mf carriers (p<0.05).

The results of the Og4C3 antigen detection test in the 30 mf carriers, from whom both filter paper and sera samples were collected are shown in Table II. The antigen positivity of filter paper samples was 93.3% for samples collected before noon (Table II). The antigen positivity rates were comparatively lower for samples collected during the afternoon (1400–1600 hours) and night (2000–2200 hours). Statistical analyses revealed that the proportion positivity was significantly different between the forenoon and afternoon (p<0.05), but not different between the forenoon and night samples (p>0.05). However, there was no significant difference in GMT of the samples collected during the 4 different time periods (p>0.05; Table II). The percentage positivity in sera samples (both during day and night) was higher (100%) compared to that of filter paper samples at any time period (p<0.05).

The GMT of mf carriers in relation to mf count was also studied. The mf counts of various individuals (n=63) were classified into 3 groups by cluster analysis and plotted against geometric mean antigenaemia titre. The results indicate that in sera samples collected during the day (1000–1200 hours) and night (2000–2200 hours) the GMT increased with an increase in the parasite count (Fig. 1). A similar increasing trend in GMT with an increasing parasite count was also observed in filter paper samples collected during the night (2000–2200 hours; Fig. 2). Regression analysis showed that there was a significant association between antigen levels (titre) and mf counts in sera samples collected during the night (R=0.137 and p=0.044) but not during the day (R=0.209 and p=0.226). However, regression analysis did not show any significant correlation in the case of filter papers, irrespective of the period of collection (for 0600–0800 hours R=0.070 and p=0.71; 1000–1200 hours, R=0.03 and p=0.875; 1400–1600 hours, R=0.173 and p=0.36 and for 2000–2200 hours, R=0.189 and p=0.317).

### Table II. Comparison of antigenaemia levels and percentage positivity of blood samples and sera samples collected on filter paper discs from 30 mf carriers during different time intervals

<table>
<thead>
<tr>
<th>Time periods (hours)</th>
<th>Geometric mean titre</th>
<th>Mean (SD) OD value</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter paper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0600–0800</td>
<td>69.18</td>
<td>0.214 (0.012)</td>
<td>28  93.3</td>
</tr>
<tr>
<td>1000–1200</td>
<td>61.8</td>
<td>0.254 (0.033)</td>
<td>28  93.3</td>
</tr>
<tr>
<td>1400–1600</td>
<td>67.76</td>
<td>0.174 (0.007)</td>
<td>23  76.7</td>
</tr>
<tr>
<td>2000–2200</td>
<td>57.67</td>
<td>0.203 (0.011)</td>
<td>26  86.7</td>
</tr>
<tr>
<td>Serum samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000–1200</td>
<td>612.35</td>
<td>0.348 (0.200)</td>
<td>30  100.0</td>
</tr>
<tr>
<td>2000–2200</td>
<td>628.05</td>
<td>0.367 (0.022)</td>
<td>30  100.0</td>
</tr>
</tbody>
</table>

### Table I. Antigenaemia levels and positivity among different groups of subjects determined by the Og4C3 ELISA for detecting Wuchereria bancrofti circulating antigens

<table>
<thead>
<tr>
<th>Group</th>
<th>Geometric mean titre</th>
<th>Mean (SD) OD value</th>
<th>Positive sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-endemic normal</td>
<td>40  11.00</td>
<td>0.104 (0.003)</td>
<td>0  0.0</td>
</tr>
<tr>
<td>Endemic normal</td>
<td>24  20.00</td>
<td>0.131 (0.011)</td>
<td>9  37.5</td>
</tr>
<tr>
<td>Microfilaria carrier</td>
<td>63  601.20</td>
<td>0.452 (0.023)</td>
<td>61  96.8</td>
</tr>
<tr>
<td>Hydrocele</td>
<td>20  23.70</td>
<td>0.131 (0.009)</td>
<td>10  50.0</td>
</tr>
<tr>
<td>Lymphoedema</td>
<td>120  18.98</td>
<td>0.121 (0.002)</td>
<td>45  37.5</td>
</tr>
<tr>
<td>Adenolymphangitis</td>
<td>39  17.70</td>
<td>0.116 (0.003)</td>
<td>14  35.8</td>
</tr>
</tbody>
</table>
DISCUSSION

The findings of the present study showed that the Og4C3 kit is efficient in detecting mf carriers in a population. Therefore, it could be useful in assessing the infection status of the community. The sensitivity and specificity of the kit was high in detecting mf carriers even when blood samples collected on filter paper were used. The high specificity of the kit recorded in the present study corroborates the findings of earlier studies.13,14 Earlier studies on night blood samples have reported a sensitivity of 75%-100%,3,14-17. The test also detected a significant proportion of endemic normal (asymptomatic and microfilaraemic) individuals as positives for CFA. This could be due to the presence of non-feudal adult worms or pre-patent infections, which cannot be detected by conventional methods. This suggests that the actual prevalence of infection in the community could be higher than that observed by conventional night blood examination, as hypothesized earlier.18

Another interesting finding was a significant difference in the sensitivity of the assay carried out on blood samples collected on filter paper during different time periods of the day. Samples collected before noon had a significantly higher percentage of positivity leading to a higher sensitivity of the assay, than those collected during the afternoon. This means that if samples are collected in the morning hours the test will not miss positive samples, especially those with a low mf count. This is important in view of the fact that sampling in the morning is more acceptable to the community than sampling at night or in the afternoon. Morning hour sample collection will also be more convenient for surveillance workers and therefore is likely to result in a better coverage than night time sampling. However, the reasons for the difference in the sensitivity of the assay on filter paper samples are not clear, particularly since there was no significant difference in the GMT at the 4 time periods. Data on parallel sera samples, if available for all the 4 time periods, would have helped to explain the results. Since all the parasite carriers were treated with a full course of diethyl carbamazine (6 mg/kg/day for 12 days), soon after the final blood sample collection, it was not possible to re-examine the cases later on.

The prevalence of anaemia among lymphoedema patients was 37.5% in the present study as against 0%-87.5% in earlier studies.3,14,16 The antigen prevalence was 50% among hydrocele patients and is similar to earlier findings.16 The chronic cases had much lower antigenaemia levels compared to microfilaraemics, suggesting that the presence of non-feudal adult worms or pre-patent infections, which cannot be detected by conventional methods. This suggests that the actual prevalence of infection in the community could be higher than that observed by conventional night blood examination, as hypothesized earlier.18

ACKNOWLEDGEMENTS

We wish to thank Dr P. K. Das, Director, and Dr K. Balaraman, Deputy Director (Senior Grade), Vector Control Research Centre for critically reviewing the manuscript. The technical assistance rendered by Mrs K. Vijayalakshmi and Mr Ramu is gratefully acknowledged. This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

REFERENCES