# Low positive predictive value of anti-*Brugia malayi* IgG and IgG4 serology for the diagnosis of *Wuchereria bancrofti*

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**Abstract**

Enzyme-linked immunosorbent assays (ELISAs) for anti-*Brugia malayi* immunoglobulin (Ig) G and $IgG_4$ were evaluated on sera from 1561 subjects in French Polynesia for the serodiagnosis of *Wuchereria bancrofti* filariasis, compared with the test for *Onchocerca gibsoni* circulating antigen (Og4C3) as a 'gold standard'. The sensitivity of the ELISA-IgG and ELISA-$IgG_4$ assays was 90·8% and 94·5%, and the specificity was 45·9% and 50·7%. The positive predictive values were 41% and 45% respectively for an antigen prevalence rate of 30%. Thus antibody prevalences exceeded by two-fold the antigen prevalence, which itself exceeded by two-fold the prevalence of microfilaraemia.

## Introduction

Anti-*Brugia malayi* immunoglobulin (Ig) G and $IgG_4$ detection tests (LAL & OTTESEN, 1988) are widely used for the diagnosis of active filariasis and for seroepidemiological studies, even though they are known to overestimate the prevalence of filarial infection. The exact evaluation of their sensitivity and specificity is hampered by the lack of a reference test (a 'gold standard') to differentiate infected and uninfected individuals in endemic populations. In French Polynesia, where *Wuchereria bancrofti* (var. *pacifica*) is the only human filaria, using microfilaraemic patients as positive controls, these 2 tests have been found to be of similar diagnostic value (CHANTEAU *et al.*, 1991). In the present study, we evaluated the tests by comparison with the test for circulating *Onchocerca gibsoni* antigen Og4C3 (MORE & COPEMAN, 1990), which has previously been demonstrated to be a marker of infection and adult parasitic burden in bancroftian filariasis (CHANTEAU *et al.*, in press).

## Methods

The study involved 1561 individuals more than 14 years old from French Polynesia and 50 subjects from non-endemic countries. Microfilaraemia was determined by filtration of 1 mL of blood collected in ethylenediaminetetraacetic acid (DESOWITZ *et al.*, 1973). Anti-*B. malayi* IgG and $IgG_4$ tests were performed on plasma, by enzyme-linked immunosorbent assays (ELISA-IgG and ELISA-$IgG_4$) using an extract of adult *B. malayi* as antigen (CHANTEAU *et al.*, 1991). IgG results were expressed in arbitrary units per mL according to a high-titre standard pool of sera, whereas $IgG_4$ results were expressed in μg per mL according to a calibrated standard. The Og4C3 thermostable circulating antigen was quantitated using an antigen capture ELISA with the monoclonal anti-*O. gibsoni* antibody Og4C3 (MORE & COPEMAN, 1990), according to the manufacturer's recommendations (JCU Tropical Biotechnology, Townsville, Australia). Soluble *O. gibsoni* extract, expressed in arbitrary antigen units, was provided as standard. The sera were scored as positive or negative according to cut-off values determined with sera from healthy Polynesian blood donors as 600 units/mL for ELISA-IgG and 4 μg/mL for ELISA-$IgG_4$ (CHANTEAU *et al.*, 1991), and the manufacturer's recommendation for the Og4C3 antigen ELISA (100 units/mL). The quantitative reproducibility of the antigen assay was assessed by the intra-class correlation coefficient (ICCC) (FERMANIAN, 1984), using data obtained from the sera of 12 individuals whose blood was sampled 12 times each.

## Results

Of the 1561 individuals tested, 221 (14·1%) were microfilaraemic, 435 (27·8%) were antigen positive, 966 (61·8%) were $IgG_4$ positive, and 1004 (64·3%) were IgG positive. Of the microfilaraemic patients (range 1–10 000 microfilariae/mL), 206 (93·2%) were antigen positive. All of the 50 sera from non-endemic areas were antigen negative. The quantitative reproducibility of the Og4C3 antigen assay was excellent (ICCC=0·994, 95% confidence interval 0·991–0·996).

The sensitivity and the specificity of the ELISA-IgG and ELISA-$IgG_4$ were analysed first independently (Table 1) and then by combining both tests (Table 2). The agreement (concordant results/total) between the ELISA-IgG and ELISA-$IgG_4$ was 84%. The predictive values for a positive result (PVP according to the theorem of BAYES (1763)) according to the prevalence of Og4C3 antigen are shown in the Figure. The PVP increased from 1·6 to 41·8% for ELISA-IgG and from 1·9 to 45·1% for ELISA-$IgG_4$ as the antigen prevalence increased from 1 to 30%. Table 3 shows the antigen prevalences according to the IgG and $IgG_4$ status of the 1561 individuals tested.

**Table 1. Sensitivity and specificity of enzyme-linked immunosorbent assays for anti-*Brugia malayi* IgG and $IgG_4$ (ELISA-IgG and ELISA-$IgG_4$) for diagnosis of *Wuchereria bancrofti* infection in French Polynesia, compared with the prevalence of circulating antigen Og4C3**[a]

| | ELISA-IgG | ELISA-$IgG_4$ |
|---|---|---|
| Sensitivity (%)[b] | 90·8 (87·7–93·2) | 94·5 (91·9–96·3) |
| Specificity (%)[b] | 45·9 (43·0–48·8) | 50·7 (47·8–53·6) |

[a]1561 sera tested (435 antigen positive, 1126 antigen negative).
[b]95% confidence intervals in parentheses.

**Table 2. Sensitivity and specificity of combined enzyme-linked immunosorbent assays for anti-*Brugia malayi* IgG and $IgG_4$ (ELISA-IgG and ELISA-$IgG_4$) for diagnosis of *Wuchereria bancrofti* infection in French Polynesia, compared with the prevalence of circulating antigen Og4C3**[a]

| | ELISA-IgG and $IgG_4$[b] | ELISA-IgG or $IgG_4$[c] |
|---|---|---|
| Sensitivity (%)[d] | 88·3 (84·9–91·0) | 97·0 (94·9–98·3) |
| Specificity (%)[d] | 57·9 (54·9–60·8) | 38·7 (35·8–41·6) |

[a]1561 sera tested (435 antigen positive, 1126 antigen negative).
[b]Combination considered positive only when both tests were simultaneously positive.
[c]Combination considered positive when at least one test was positive.
[d]95% confidence intervals in parentheses.

## Discussion

The Og4C3 antigen capture assay has been shown to be very specific for the diagnosis of bancroftian filariasis (MORE & COPEMAN, 1990). Recently, using several indi-

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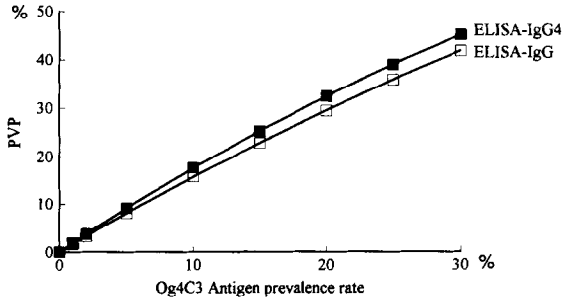


Figure. Predictive value for a positive result (PVP) using enzyme-linked immunosorbent assays for anti-*Brugia malayi* IgG and $IgG_4$ (ELISA-IgG and ELISA-$IgG_4$) to diagnose *Wuchereria bancrofti* infection in 1561 inhabitants of French Polynesia, compared with the prevalence of Og4C3 antigenaemia.

**Table 3. Prevalence rate of antigen Og4C3 in 1561 inhabitants of French Polynesia according to their anti-*Brugia malayi* IgG and $IgG_4$ status as determined by enzyme-linked immunosorbent assay (ELISA-IgG and ELISA-$IgG_4$)**

| | ELISA-IgG | | | |
|---|---|---|---|---|
| | Positive ($n$=1004)[a] | | Negative ($n$=557) | |
| | ELISA-$IgG_4$ | | ELISA-$IgG_4$ | |
| | Positive | Negative | Positive | Negative |
| | 858/1004[b] (85%) | 146/1004 (15%) | 108/557[c] (19%) | 449/557 (81%) |
| Og4C3 | | | | |
| No. positive | 384/858 (45%) | 11/146 (8%) | 27/108 (25%) | 13/449 (3%) |
| Geometric mean (units/mL) | 31 | 1 | 6 | 0·1 |

[a]Geometric mean antibody level (GM)=2943 units/mL.
[b]GM=51 μg/mL.
[c]GM=9 μg/mL.

rect experiments, the macrofilarial origin of this antigen was demonstrated and it was concluded that the test can be used as a marker of *W. bancrofti* infection (CHANTEAU *et al.*, in press). The sensitivity of both the ELISA-IgG and ELISA-$IgG_4$ was very high (90·8% and 94·5% respectively), whereas the specificity was notably low (45·9% and 50·7% respectively). Consequently, the predictive value of a positive result was also low: fewer than half of the individuals scored as positive by the IgG or $IgG_4$ ELISAs were actually infected. Antibody prevalences exceeded two-fold the antigen prevalence, which itself exceeded two-fold the prevalence of microfilaraemia. In Papua New Guinea, a similar ratio was observed between antigen prevalence (57%) and prevalence of microfilaraemia (26%) (TURNER *et al.*, in press). The discrepancy between antibody and antigen detection tests in sera from endemic populations may be attributed to the persistence of antibodies after infection, to frequent aborted filarial infections, and to cross-reactivity between antigens from other parasites. Increasing the specificity of the antibody tests by increasing their cut-off values would decrease their sensitivities. The use of specific recombinant antigen would be a possibility in the future. Compared with the Og4C3 antigen test, the ELISA-$IgG_4$ had slightly better sensitivity and specificity than the ELISA-IgG. This was confirmed by the higher antigen prevalence observed in the $IgG_4$ positive individuals whatever their IgG status.

In conclusion, the anti-filarial IgG and $IgG_4$ ELISAs were found to be very sensitive but not specific enough when compared to Og4C3 antigenaemia. As a result, their positive predictive values for *W. bancrofti* infection were very low.

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