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Density-dependent reactivity of the STANDARD Q Filariasis Antigen Test in individuals with Loa loa microfilaraemia in an area with no evidence of lymphatic filariasis in Gabon : a cross-sectional study

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Short Report

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Abstract

Background

Lymphatic filariasis (LF) programmes increasingly rely on the STANDARD Q Filariasis Antigen Test (QFAT) to detect *Wuchereria bancrofti* circulating filarial antigen. Older antigen tests cross-react in people with heavy *Loa loa* microfilaraemia, which complicates LF mapping in Central Africa. Field evaluations of QFAT have so far been conducted outside loiasis-endemic areas, and recent confirmatory mapping in Gabon found antigen-positive children without *W. bancrofti* infection.

Methods

We performed QFAT on stored plasma samples (− 30°C, one freeze–thaw cycle) collected within the PHYLECOG project from 50 adults with microscopically confirmed *L. loa* microfilaraemia in the Ntem and Haut-Ntem departments, Woleu-Ntem province, northern Gabon. Microfilarial density was quantified by direct examination and leukoconcentration of venous blood collected between 10:00 and 15:00. We compared density between QFAT-positive and QFAT-negative participants and modelled the probability of a positive test as a function of log-transformed density.

Results

Twelve of 50 participants (24.0%; 95% confidence interval [CI] 14.3–37.4%) were QFAT-positive. No participant below 3,000 microfilariae (mf)/mL was positive (0/30); positivity was 14.3% (1/7) at 3,000–8,000 mf/mL, 75.0% (6/8) at > 8,000–30,000 mf/mL and 100% (5/5) above 30,000 mf/mL. Eleven of 13 hypermicrofilaraemic participants (≥ 8,000 mf/mL; 84.6%) were QFAT-positive, compared with 1 of 37 (2.7%) below this threshold. Median microfilaraemia was 21,300 mf/mL in QFAT-positive and 600 mf/mL in QFAT-negative participants ($P < 0.001$). Each tenfold increase in microfilaraemia was associated with higher odds of a positive QFAT (odds ratio adjusted for age and sex 105; 95% CI 5.2–2,105), and the modelled probability of positivity reached 50% at about 9,800 mf/mL. Microfilaraemia discriminated QFAT status well (area under the curve 0.97; 95% CI 0.92–1.00), and no participant below 4,900 mf/mL was positive. All three participants with *Strongyloides stercoralis* were QFAT-positive, but all three were also hypermicrofilaraemic. The four participants co-infected with *Mansonella perstans* were QFAT-negative.

Conclusions

QFAT reacts in a density-dependent manner in people with *L. loa* microfilaraemia and is mostly positive in hypermicrofilaraemic individuals, as its predecessors are. Because *W. bancrofti* was not investigated, these results describe reactivity, not diagnostic accuracy. In loiasis co-endemic areas, QFAT-positive

results should be confirmed by nocturnal *W. bancrofti* microscopy or molecular testing and interpreted alongside daytime *L. loa* quantification before they inform programme decisions.

Background

Lymphatic filariasis (LF) programmes rely on rapid tests that detect circulating filarial antigen (CFA) of *Wuchereria bancrofti* to decide where mass drug administration (MDA) is needed [1, 2]. Since 2013, the Filariasis Test Strip (FTS) has been the main point-of-care test [3]. In Central Africa, this approach is complicated by loiasis. Filarial antigenaemia without *W. bancrofti* infection was first described in the Democratic Republic of the Congo in people with *Loa loa* microfilaraemia [4]. The BinaxNOW Filariasis immunochromatographic card test (ICT) and FTS were later shown to be positive in individuals with high *L. loa* microfilaraemia [5–7], and the *L. loa* antigens responsible for this cross-reactivity have been identified [8]. In Cameroon, confirmatory strategies that accounted for loiasis found no evidence of *W. bancrofti* endemicity in areas first flagged by antigen testing [9, 10]. In southern Gabon, FTS positivity during integrated mapping rose from 4.3% in individuals with 1–10,000 microfilariae (mf)/mL to 52.9% in those with more than 30,000 mf/mL [11].

The STANDARD™ Q Filariasis Antigen Test (QFAT; SD Biosensor) is a cassette-format alternative to FTS. Field comparisons in India and Samoa showed near-perfect agreement with FTS [12, 13], and a laboratory comparison found similar analytical performance [14]. Both field evaluations were conducted outside loiasis-endemic areas. Dinesh et al. noted that QFAT cross-reactivity with *L. loa* remained to be assessed [12], whereas Scott et al. considered it unlikely on the basis of laboratory testing [13, 14].

The question has become practical in Gabon. QFAT was recently used for LF confirmatory mapping of 6,688 children aged 9–14 years in 18 health departments: 13 children (0.19%) were QFAT-positive, none had *W. bancrofti* microfilariae or DNA, and *L. loa* was found in three of them [15]. To our knowledge, QFAT results in individuals with quantified *L. loa* microfilaraemia have not been reported. We therefore describe QFAT reactivity according to *L. loa* microfilaraemia in adults from a loiasis-hyperendemic area of northern Gabon. This Brief Report extends the field evaluations of QFAT [12, 13] to a loiasis-endemic setting.

Methods

Study area and samples. This cross-sectional study used stored plasma samples collected within the PHYLECOG project (EDCTP2, TMA2019CDF-2730; trial registration : ISRCTN14889921), which enrolled adults with *L. loa* microfilaraemia in villages around Bitam (Ntem department) and Minvoul (Haut-Ntem department), Woleu-Ntem province, northern Gabon, between November 2021 and April 2022 [16, 17]. In this rural forest area, the prevalence of microfilaraemic loiasis is about 20% [18, 19]. Neither department was among the 18 health departments retained for LF confirmatory mapping in 2025, which confirmed that Gabon is not endemic for LF [15, 20]. Samples from 50 participants with microscopically confirmed *L. loa* microfilaraemia, from 15 villages of the Ntem (n = 36) and Haut-Ntem (n = 14) departments, were

tested with QFAT. All PHYLECOG participants with *L. loa* microfilaraemia and an available plasma aliquot were first listed. Samples were then selected at random within classes of microfilaraemia (< 100, 100 to < 3,000, 3,000–8,000, > 8,000–30,000 and > 30,000 mf/mL), so that the full range of densities was represented.

Blood collection and parasitological examinations. Venous blood (5 mL) was collected in EDTA tubes between 10:00 and 15:00, to match the diurnal periodicity of *L. loa*. *L. loa* and *Mansonella perstans* microfilaraemia were determined by direct examination of 10 µL of fresh blood, with serial tenfold dilutions for high densities, and by the leukoconcentration technique of Ho Thi Sang and Petithory on 5 mL of blood when the direct examination was negative [17, 21, 22]. Microfilaraemia was expressed as microfilariae per millilitre of blood (mf/mL), and hypermicrofilaraemia was defined as ≥ 8,000 mf/mL [16, 23]. Stool samples, when provided, were examined by the Kato-Katz technique, merthiolate-iodine-formaldehyde (MIF) staining and concentration, and coproculture for *Strongyloides stercoralis* larvae. *Plasmodium* infection was assessed by Giemsa-stained thick blood smear. Clinical manifestations of loiasis (Calabar swelling, eye worm, crawling sensation and pruritus) were recorded with a standard questionnaire.

Plasma preparation and storage. After centrifugation of the EDTA whole blood, plasma was aliquoted into labelled cryotubes and stored at − 30°C. Each aliquot underwent a single freeze–thaw cycle, for the purpose of this study.

QFAT. All samples were tested in a single session in September 2026 with the STANDARD™ Q Filariasis Antigen Test (SD Biosensor, Suwon, Republic of Korea; REF QFIL01G; lot 51J64G1D1; buffer lot R/FLSAG/250416-1; expiry 15 April 2027), using two kits of 25 tests provided by the Programme National de Lutte contre les Maladies Parasitaires (PNLMP), Gabon. Each test was performed on 10 µL of thawed plasma with chase buffer, following the manufacturer's procedure for serum and plasma. Results were read at 20 min, the upper limit of the manufacturer's 10–20-min reading window, independently by two readers unaware of microfilaraemia. Any visible test line was considered positive. Each sample was tested once, and all 50 tests gave a valid result.

Statistical analysis. Proportions are given with Wilson 95% confidence intervals (CIs). Microfilaraemia was compared between QFAT-positive and QFAT-negative participants with the Mann–Whitney *U* test, and categorical variables with Fisher's exact test, the chi-square test or the Cochran–Armitage test for trend. The association between log10-transformed microfilaraemia and QFAT positivity was estimated by logistic regression, crude and adjusted for age and sex; the crude model was used to derive the densities associated with 50% and 90% probability of positivity. Discrimination was assessed by the area under the receiver operating characteristic (ROC) curve (AUC), with a bootstrap 95% CI (2,000 resamples), and the optimal threshold was chosen with the Youden index. A sensitivity analysis excluded participants with *S. stercoralis*. Analyses were performed in Python 3 (SciPy, statsmodels and scikit-learn). $P < 0.05$ was considered significant.

Results

The 50 participants had a median age of 54.5 years (interquartile range [IQR] 41.5–61.8; range 18–88); 30 (60.0%) were men and 36 (72.0%) lived in Ntem department. Median microfilaraemia was 1,300 mf/mL (IQR 100–7,625; range 1–105,900), and 13 participants (26.0%) were hypermicrofilaraemic. Twelve participants (24.0%; 95% CI 14.3–37.4%) were QFAT-positive. QFAT positivity was not associated with sex, department (Table 1) or age group (18–44 years, 2/15; 45–59 years, 7/18; ≥60 years, 3/17; P = 0.17) (Table 1).

Table 1
Characteristics of participants with *L. loa* microfilaraemia by QFAT result

Characteristic	**QFAT-positive (*n* = 12)**	**QFAT-negative (*n* = 38)**	***P***
Age, years, median (IQR)	51.5 (47.8–60.8)	55.0 (39.5–61.8)	0.72
Male sex, *n* (%)	6 (50.0)	24 (63.2)	0.51
Ntem department, *n* (%)	8 (66.7)	28 (73.7)	0.72
L. loa density, mf/mL, median (IQR)	21,300 (13,250–46,775)	600 (7–2,150)	< 0.001
L. loa density, mf/mL, range	4,900–105,900	1–26,400	—
M. perstans co-infection, *n* (%)	0 (0)	4 (10.5)	0.56
Plasmodium spp. on thick smear, *n* (%)	2 (16.7)	9 (23.7)	1.00
Stool examined, *n*	8	29	—
S. stercoralis, *n*/examined (%)	3/8 (37.5)	0/29 (0)	0.007
Hookworm, *n*/examined (%)	0/8 (0)	3/29 (10.3)	1.00
Ascaris lumbricoides, *n*/examined (%)	1/8 (12.5)	4/29 (13.8)	1.00
Trichuris trichiura, *n*/examined (%)	1/8 (12.5)	5/29 (17.2)	1.00

 Mann–Whitney *U* test; Fisher's exact test. IQR, interquartile range; mf, microfilariae.

QFAT positivity increased sharply with *L. loa* microfilaraemia (Table 2; P for trend < 0.001). None of the 30 participants below 3,000 mf/mL was positive, whereas all five above 30,000 mf/mL were. Overall, 11 of 13 hypermicrofilaraemic participants (84.6%; 95% CI 57.8–95.7%) were QFAT-positive, compared with 1 of 37 (2.7%; 95% CI 0.5–13.8%) below 8,000 mf/mL (P < 0.001). Median microfilaraemia was 21,300 mf/mL in QFAT-positive and 600 mf/mL in QFAT-negative participants (P < 0.001); geometric means were 24,523 and 181 mf/mL, respectively.

Table 2
QFAT positivity by *L. loa* microfilarial density

***L. loa* microfilaraemia (mf/mL)**	***n***	**QFAT-positive, *n* (%)**
< 100	11	0 (0)
100 to < 3,000	19	0 (0)
3,000–8,000	7	1 (14.3)
> 8,000–30,000	8	6 (75.0)
> 30,000	5	5 (100)
Hypermicrofilaraemia (≥ 8,000)	13	11 (84.6)
Below 8,000	37	1 (2.7)
Total	**50**	**12 (24.0)**

Each tenfold increase in microfilaraemia was associated with higher odds of QFAT positivity (crude odds ratio [OR] 139; 95% CI 4.9–3,944; *P* = 0.004; OR adjusted for age and sex 105; 95% CI 5.2–2,105; *P* = 0.002). The modelled probability of a positive QFAT was 39% at 8,000 mf/mL, reached 50% at about 9,800 mf/mL and was 92% at 30,000 mf/mL (Fig. 1). Microfilaraemia discriminated QFAT status well (AUC 0.97; 95% CI 0.92–1.00). At the Youden-optimal threshold of 4,900 mf/mL, all QFAT-positive participants were at or above the threshold (sensitivity 100%) and 33 of 38 QFAT-negative participants (86.8%) were below it. Two hypermicrofilaraemic participants (13,700 and 26,400 mf/mL) were QFAT-negative.

All three participants with *S. stercoralis* (among 37 with stool results) were QFAT-positive (*P* = 0.007), but all three were hypermicrofilaraemic (8,100, 40,300 and 86,000 mf/mL). Excluding them did not change the association with microfilaraemia (OR 139; 95% CI 3.9-4,924; AUC = 0.97), and 8 of the 10 remaining hypermicrofilaraemic participants were QFAT-positive. The four participants co-infected with *M. perstans* were QFAT-negative, but their *L. loa* microfilaraemia was low (≤ 4,900 mf/mL). Clinical data were available for 20 participants; Calabar swelling (n = 2), eye worm (n = 2), crawling sensation (n = 2) and pruritus (n = 4) were too infrequent for comparison by QFAT status.

Discussion

In this first report of QFAT results in people with quantified *L. loa* microfilaraemia, one in four tested positive, and positivity depended strongly on microfilarial density: none below 3,000 mf/mL, about half near 10,000 mf/mL and all above 30,000 mf/mL. This pattern mirrors what was reported for ICT and FTS in Cameroon and Gabon [5, 6, 11]. In the integrated mapping survey of southern Gabon, FTS was positive in 52.9% of individuals with more than 30,000 mf/mL [11]; in our series, QFAT was positive in all five. Populations, sample types and microscopy methods differ between the two studies, but these data suggest that QFAT is at least as reactive as FTS in people with high *L. loa* microfilaraemia.

The most likely explanation is cross-reactivity with *L. loa* antigens circulating in heavily infected hosts. Hertz et al. identified the *L. loa* antigens responsible for cross-reactivity with ICT and FTS [8]. The QFAT capture antibody is not publicly described, but our findings suggest that it recognises a similar epitope, or that *L. loa* antigen load becomes detectable at high density. A density-dependent signal is more consistent with antigen cross-reactivity than with undetected *W. bancrofti* infection, which would not be expected to track *L. loa* density.

These findings help interpret the recent confirmatory mapping in Gabon, where none of the 13 QFAT-positive children had *W. bancrofti* microfilariae or DNA [15]. The *L. loa* densities found in those children were low (1–10 microfilariae per 60-µL smear), which appears at odds with a density-dependent mechanism [15]. However, those smears were collected between 22:00 and 01:00 to detect nocturnal *W. bancrofti*. *L. loa* microfilaraemia follows a diurnal periodicity, so night-time counts probably underestimate the true parasite burden. In our study, microfilaraemia was measured between 10:00 and 15:00 with leukoconcentration of 5 mL of blood, which detects low densities missed by thick blood smears [21]. Nine of the 13 QFAT-positive children had no microfilariae at night [15]; daytime quantification would be needed to know whether they carried *L. loa*. The gap between 0.19% positivity among children in the national survey and 24.0% in our microfilaraemic adults is also consistent with the lower prevalence and intensity of *L. loa* infection at younger ages [11].

The role of *S. stercoralis* cannot be settled with these data. All three infected participants were QFAT-positive, but they were also hypermicrofilaraemic, and the association with microfilaraemia remained after excluding them. A laboratory evaluation did not find QFAT cross-reactivity with human *Strongyloides* infection, although it did find reactivity with two canine filariae [14]. Concerns about *Strongyloides* had previously been raised for FTS [3]. *M. perstans* co-infection was not associated with QFAT positivity, but all four co-infected participants had low *L. loa* microfilaraemia.

This study has limitations. First, *W. bancrofti* was not investigated, and no *W. bancrofti*-infected or *L. loa*-negative controls were tested. The results therefore describe QFAT reactivity in loiasis and cannot be used to estimate QFAT sensitivity or specificity for LF. *W. bancrofti* co-infection is unlikely, given that national confirmatory mapping conducted in 2025 found no evidence supporting LF endemicity in Gabon [15, 20]; however, it cannot be excluded in individual participants because *W. bancrofti*-specific testing was not performed in the present study. Second, QFAT is intended for capillary whole blood at the point of care. We used plasma stored at − 30°C after one freeze–thaw cycle, as in the laboratory evaluation of QFAT [14]; antigen is concentrated in plasma, and storage may alter detection, so reactivity in the field could differ. Third, reading at 20 min may increase positivity compared with reading at 10 min [12]. Fourth, the sample was small, with only five participants above 30,000 mf/mL; the CIs around the odds ratios are therefore wide. Because sampling was stratified by microfilaraemia, the overall positivity of 24.0% reflects our sampling design and is not an estimate of QFAT positivity among all microfilaraemic adults in the area. Fifth, stool was examined in 37 participants only, and other filariae such as *Onchocerca volvulus* were not investigated.

Despite these limitations, the findings have practical implications. A single antigen-positive result counts toward the LF confirmatory mapping threshold, and three positives can place a health department above it [2, 15]. Woleu-Ntem and many other areas of Gabon are hyperendemic for loiasis, with hypermicrofilaraemia in a substantial proportion of infected adults [11, 16]. QFAT-positive results in these settings should not be interpreted as *W. bancrofti* infection without confirmation. Programmes could quantify *L. loa* by daytime microscopy at the time of antigen testing, and confirm *W. bancrofti* infection by nocturnal microscopy and Wb-LDR qPCR, as done in the national survey [15]. WHO guidance for LF elimination in loiasis-endemic countries already recognises this diagnostic challenge [24]. QFAT should now be evaluated on well-characterised panels that include *W. bancrofti*, *L. loa* across the full range of microfilaraemia, *M. perstans*, *O. volvulus* and *S. stercoralis* infections, and uninfected controls.

Conclusions

QFAT reactivity in Gabonese adults with loiasis was strongly associated with *L. loa* microfilaraemia and concentrated among hypermicrofilaraemic individuals, with positivity becoming likely above about 10,000 mf/mL. Like its predecessors, QFAT cannot on its own distinguish *W. bancrofti* infection from heavy loiasis. In loiasis co-endemic areas, a positive QFAT should prompt *L. loa* quantification and *W. bancrofti*-specific confirmation before it informs LF programme decisions.

Abbreviations

AUC
area under the receiver operating characteristic curve
CFA
circulating filarial antigen
CI
confidence interval
EDTA
ethylenediaminetetraacetic acid
FTS
Filariasis Test Strip
ICT
immunochromatographic card test
IQR
interquartile range
LF
lymphatic filariasis
MDA
mass drug administration
mf
microfilariae

MIF
merthiolate-iodine-formaldehyde
OR
odds ratio
PNLMP
Programme National de Lutte contre les Maladies Parasitaires
qPCR
quantitative polymerase chain reaction
QFAT
STANDARD™ Q Filariasis Antigen Test
ROC
receiver operating characteristic
Wb-LDR
W. bancrofti long DNA repeat.

Declarations

Ethics approval and consent to participate. This study used samples collected within the PHYLECOG project. The protocol was approved by the National Ethics Committee for Scientific Research of Gabon (CNER; Protocol No. 0053/2022/CNER/P/SG) and registered under ISRCTN14889921. Written informed consent was obtained from each participant, including consent to the reuse of their samples and data for further research. Authorisation was also obtained from local health authorities and village chiefs.

Consent for publication. Not applicable.

Availability of data and materials. The dataset used and analysed during the current study is available from the corresponding author on reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. NPM conceived the study, performed the analysis and drafted the manuscript. LNA, AI, BPN and RMM collected the samples, performed the parasitological examinations, and performed and read the QFAT tests. JA provided the QFAT kits and reviewed the manuscript. DPM-M and MKB-A supervised the work and revised the manuscript. All authors read and approved the final manuscript.

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Graphical abstract. Landscape image (~920 × 300 px, PNG) adapted from Table 2 and Fig. 1.

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Figures

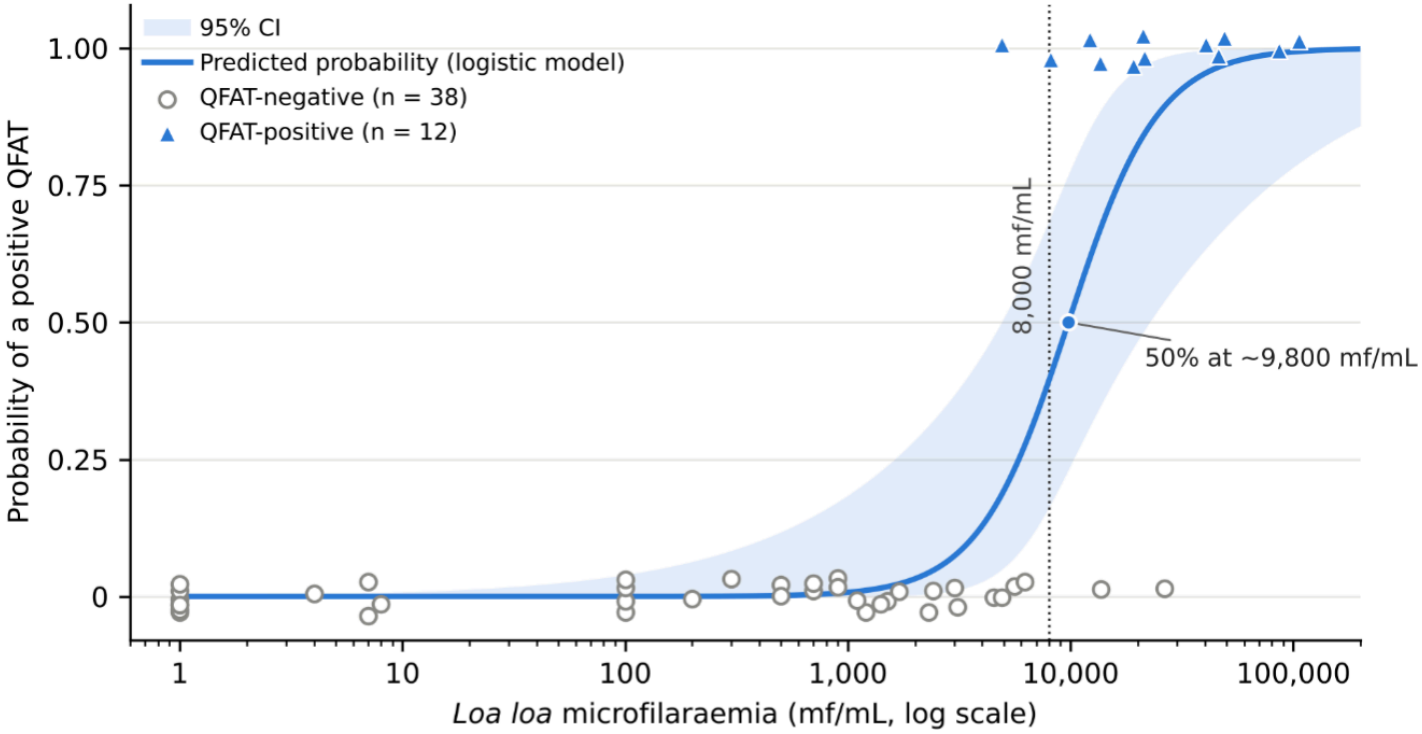


Figure 1

QFAT result by *L. loa* microfilarial density in 50 microfilaraemic adults from the Ntem and Haut-Ntem departments, Woleu-Ntem province, northern Gabon. Each symbol is one participant (top row, QFAT-positive, filled triangles; bottom row, QFAT-negative, open circles; small vertical jitter added). The curve shows the probability of a positive QFAT predicted by logistic regression on log10 microfilarial density, with its 95% confidence interval (shaded band). The dotted vertical line marks the hypermicrofilaraemia

threshold (8,000 mf/mL), and the dot on the curve marks 50% predicted probability (about 9,800 mf/mL). mf, microfilariae; QFAT, STANDARD™ Q Filariasis Antigen Test.