

Studies of the Soluble Antigen Fluorescent Antibody (SAFA) Test for Filariasis

Principal Investigator : Carter L. Diggs, LTC, MC

Assistant Investigators : Stanley W. Theune, MSG
Robert Gentzel, SSG
Prasit Sookto
Nipon Chuanak

OBJECTIVE: In spite of extensive investigation, serologic reactivity in human filariasis is not sufficiently well understood to allow meaningful interpretations of serologic tests. (For Review, see 1). One of the difficulties is the possibility of serologic reactivity induced by other helminths including filarial worms not ordinarily pathogenic in man. Recently a soluble antigen fluorescent antibody (SAFA) test for bancroftian filariasis and onchocerciasis has been described². The test employs a soluble antigen derived from the dog heartworm, *Dirofilaria immitis*. The possibility has been raised that humans exposed to *D. immitis* might become seroreactive in tests employing antigens derived from the parasite.³ The present study was designed to evaluate the SAFA test for reactivity in filariasis due to *Brugia malayi* and to attempt to gain some insight regarding serologic reactivity in a population exposed to *D. immitis* infected mosquitoes. In addition, some technical characteristics of the test system were studied.

DESCRIPTION: Some parts of the test procedure were different from that previously described². Rabbit antiserum to human globulin was prepared using commercial immune serum globulin as antigen. Based on preliminary qualitative precipitin tests, pools were prepared, the globulin fraction isolated and labelled with fluorescein isothiocyanate under standard conditions. Details of these procedures will be presented in a later report. Free fluorescein was removed by chromatography on G-25 Sephadex and the antibody content of the labelled antiglobulin estimated by quantitative precipitin tests.

In performing the SAFA tests the fluorometer was zeroed by exclusion of light from the photocell. For each serum tested, a disc impregnated with antigen and a control disc without antigen were exposed to the serum. In this way nonspecific fluorescence for each serum sample was measured; antigen dependent fluorescence was taken as the difference between the control and experimental discs. The procedure allowed estimates of variation among sera in control as well as in infected populations.

Instrumental sensitivity, stability and linearity were evaluated by the use of discs treated with known amounts of fluorescein isothiocyanate.

PROGRESS: Instrumental stability, as determined by repeated measurements of the intensity of fluorescence of standard fluorescein isothiocyanate impregnated discs, was judged to be good. Linearity of response with concentration, however, was limited to about 40 fluorescence units, a value much lower than that obtained with many samples in the serologic test. Assuming that a similar nonlinearity exists for the serologic test, these results suggest that fluorescence intensities can be quantitatively related to antibody concentration only in a limited range of measurements.

Three groups of sera have been studied: (1) 128 sera from patients with *B. malayi* microfilaremia; (2) 69 sera from American soldiers recently arrived in Southeast Asia; and (3) 97 sera from Bangkokians (blood donors and patients receiving prenatal care). This latter group comprises an appropriate population for study in connection with the question of seroreactivity induced by exposure to *D. immitis* since there is active transmission of the parasite in Bangkok. Examination of the distribution of fluorescence intensities obtained revealed fairly extensive deviations from normality. The distributions are presented in Fig. 1, in which the percentage cumulative frequencies of given fluorescent intensities have been plotted on log-probit paper. It is apparent that the distribution of the transformed data approximates normality as judged from

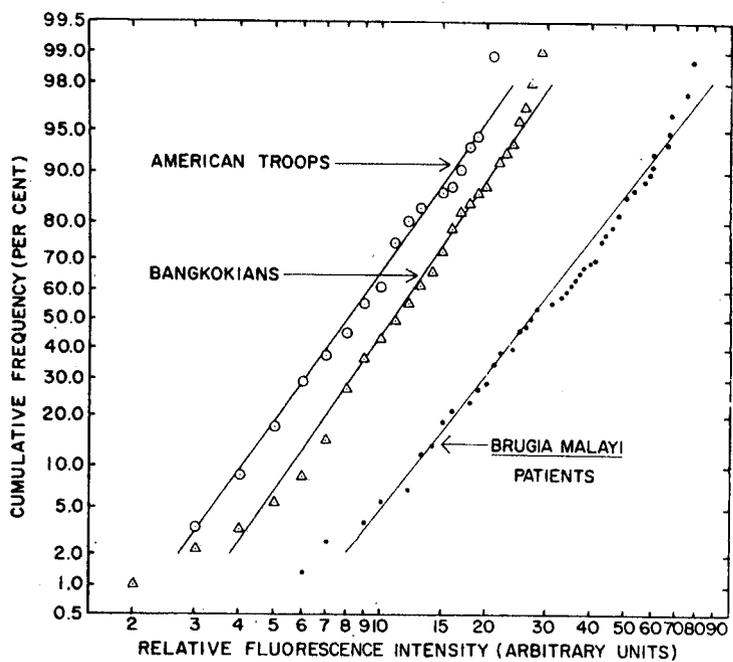


Fig 1. Log-probit plot of the cumulative frequency (probit scale) of fluorescence intensities (log scale) obtained with three groups of sera tested by the SAFA test using D. immitis antigen.

these plots. The data clearly indicate that the patients with Brugia malayi infections have a higher reactivity as a group than do either the American troops or Bangkokians. However, it is also apparent that approximately 50% of the sera from infected individuals have seroreactivities which overlap the values of the control group. Thus the usefulness, for diagnostic purposes, of the test as performed is limited. This circumstance might be related to the stage of the disease or age of the individual patient.² Alternatively, greater discrimination might be obtained through the use of higher concentrations of antigen and/or antiglobulin in the test. These possibilities are being explored.

The mean reactivity of Bangkokians was higher than that of American troops, and the difference was statistically significant ($P < 0.001$, test). However, the reactivity is not striking when compared with the filariasis group. Determination of whether or not the difference reflects antibody in the Bangkok population will require further study.

The results indicate, however, that living in an area endemic for canine heartworm does not necessarily confer seroreactivity to D. immitis antigen.

SUMMARY: The reactivity of sera from patients with Brugia malayi infections in a soluble antigen fluorescent antibody (SAFA) test using Dirofilaria immitis antigen has been studied. Approximately 50% of the patients' sera gave greater reactivity than any of the control sera obtained from Americans or Bangkok residents. The mean reactivity of the Bangkok sera was higher than that of Americans, but not markedly so. The question of seroreactivity to D. immitis antigens in human populations at risk of exposure to this parasite is therefore not resolved, but the data indicates that such seroreactivity, if it exists, does not necessarily interfere markedly with serologic testing for human filariasis in populations at risk with respect to infection by D. immitis.

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(2) Duxbury, R.E. and Sadun, E.H., Exp. Parasit. 20: 77, 1967.
(3) Garcia, E.G. et al., Jour P.M.A., 44: 149, 1968.