

Specific Antibody Levels in Individuals from a Malarious Region  
as Estimated by Radioimmunoassay

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**OBJECTIVE:** To refine a radioimmunoassay for antimalarial antibody and to evaluate the assay using sera collected from a malarious area.

**BACKGROUND:** A solid-phase radioimmunoassay has been developed for estimation of immunoglobulin class-specific antimalarial antibody (1). The assay was refined by replacing the sheep erythrocyte as an immunoadsorbent to remove the antibody from the test serum. The assay proved to be sensitive and the reproducibility was high; therefore, it became necessary to evaluate it using individual sera collected from persons in a malarious region. This study describes the results of such an evaluation.

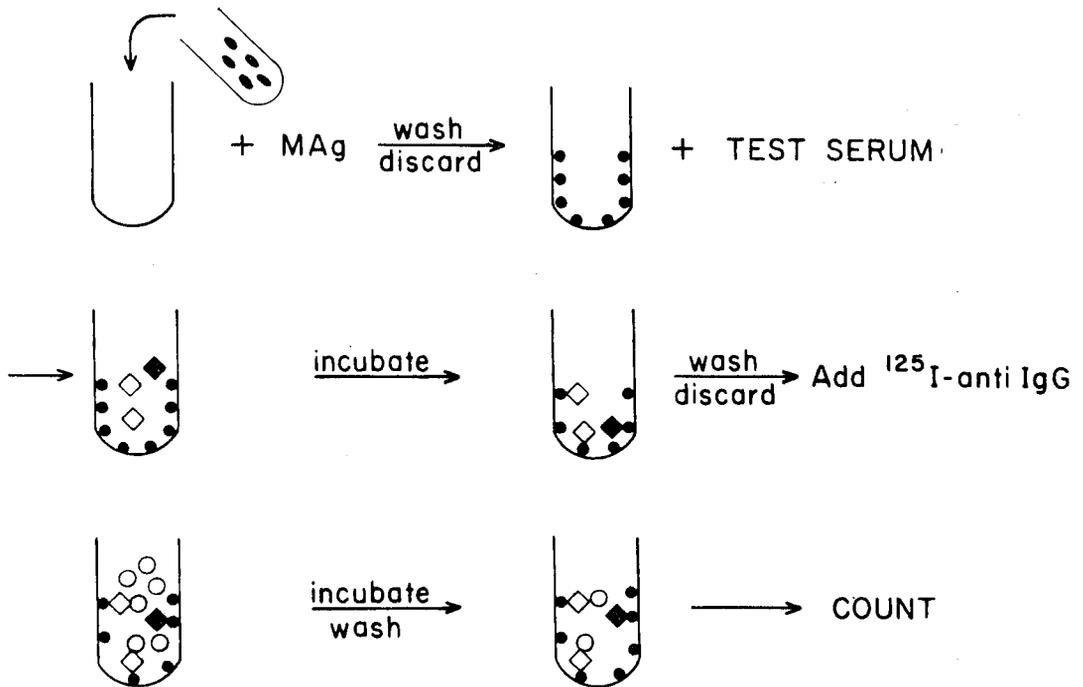
**DESCRIPTION:** The radioimmunoassay (RIA) was performed as previously described (2) with the following modifications. The third phase of the assay, the competitive binding portion, was replaced with a direct binding system as illustrated in Figure 1. Briefly, the tube containing the bound antigen-antibody complex was washed 5 times with PBS, pH 7.2; and  $^{125}\text{I}$ -labelled, purified, rabbit antihuman IgG antibody was added ( $32 \mu\text{g/ml}$ ) and allowed to incubate for 3 hours. After incubation, the antibody was aspirated and the tube washed 5 times, or until the wash no longer contained radioactivity. The tube was then counted in a gamma spectrometer. The quantity of specific anti-IgG antibody present in the test sera was estimated by comparing results from the test sera with those obtained from a standard curve developed by substituting purified human IgG for the antigen-antibody complex.

Purified anti-human IgG antibody was produced in the following manner. Human IgG, purified by batch DEAE procedures, was injected into a goat and the serum retrieved was shown to contain precipitins by immunoelectrophoresis and gel-diffusion. The antibody was purified by ammonium sulphate precipitation and by passing the dissolved precipitate across an immunoadsorbent column of human IgG linked to sepharose with cyanogen bromide. The antibody was eluted with glycine-HCl, pH 2.3 with 10% dioxane and dialyzed overnight in TRIS buffer, pH 8.0. The antibody was rendered monospecific by passing the dialyzed material through another immunoadsorbent column of myeloma IgM linked to sepharose with cyanogen bromide. Monospecificity was demonstrated with immunoelectrophoresis and radioimmuno-electrophoresis.

All sera were assayed a minimum of 2 times with duplicate preparations in each assay. Control positive serum and normal serum were included in each assay and if deviations from the mean for these control sera were too great, as determined by a quality control system (3), that particular assay was considered invalid and the data were not included.

**RESULTS:** Repeated experiments testing the amount of  $^{125}\text{I}$ -antigen and purified  $^{125}\text{I}$ -IgG bound to the polypropylene tube indicated that the amount of malarial antigen or human IgG bound to the tube was consistent in each assay with less than 1% error between assays. This provided the basis for developing the  $^{125}\text{I}$ -labelled purified antibody system described above. Retesting of the same sera after the initial adsorption demonstrated that no detectable quantities of IgG antibody could be found. 262 serum samples were assayed with 179 demonstrating detectable antibody (Table 1). The mean antibody level of the 179 individuals was  $27.07 \mu\text{g/ml}$ . Individuals who had malaria, as demonstrated by examination of stained blood smears, had a mean of  $9.36 \mu\text{g/ml}$  with 27 of 59 having detectable antibody (Table 2). A total of 17 had palpable spleens, but no correlation was observed between detectable antibody and spleen rate. Antibody levels and the infection rate by age group are presented in Figure 2.

FIGURE 1 : SCHEMATIC REPRESENTATION OF RADIOIMMUNOASSAY.



- Malaria antigen
- ◇ IgG antibody
- ◆ IgM antibody
- $^{125}\text{I}$ -labelled anti-human IgG antibody

FIGURE 2. ANTIBODY (IgG) LEVELS AND PER CENT INFECTED INDIVIDUALS

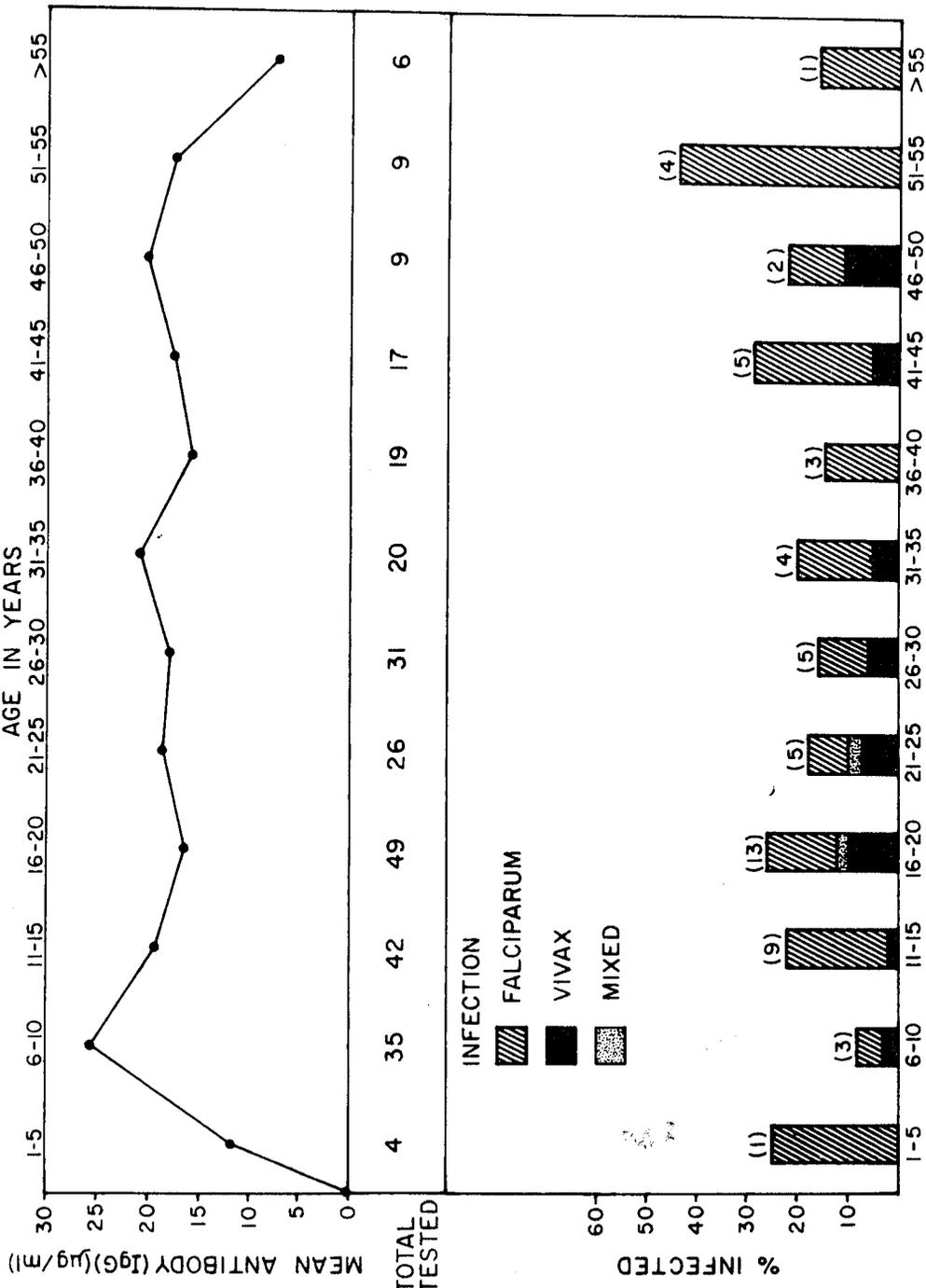


Table 1. Specific Antibody (IgG) Levels in Individuals Living in a Malarious Region of Thailand

Age	Sex	Number of Samples	Number with Antibody	Mean Antibody Level ( $\mu\text{g/ml}$ )	Range ( $\mu\text{g/ml}$ )
1-10	M	24	20	28.4	6.2-79.4
	F	16	14	25.0	0.8-52.0
11-15	M	22	17	25.4	2.4-56.0
	F	20	12	30.8	2.0-75.0
16-25	M	35	23	27.0	4.0-54.8
	F	30	18	29.3	0.4-69.6
25-40	M	42	28	30.2	0.8-81.4
	F	33	23	21.7	0.6-72.4
40+	M	27	13	20.7	8.2-54.2
	F	13	11	24.9	8.6-91.4
All males		150	101	27.07	0.8-81.4
Total		262	179	27.07	0.6-91.4

Table 2. Mean Antibody (IgG) Levels in Infected Individuals

Species	Number	With Detectable Antibody	Mean Antibody Levels $\mu\text{g/ml}$	Number with Palpable Spleens
<i>P. falciparum</i>	34	18	10.29	14
<i>P. vivax</i>	13	8	15.25	3
Mixed	2	1	2.1	0
TOTAL	59	27	9.36	17

**DISCUSSION:** The modified RIA system has proven to be as sensitive and as accurate as the previously used inhibition system and the time required to perform the assay has been reduced by approximately 50%. Serum antibody levels to this antigen were detectable in 179 of 262 samples assayed (68%). Antibody was detected in all age groups assayed with a maximum peak in the 6-10 age group, which also had the lowest rate of infection for *P. falciparum* (Fig. 2). Of the infected group, 22 of 59 (37%) did not have detectable antibody to this antigen. Data are insufficient to make any correlations between the presence of the antibody to the antigen used and immunity to malaria; however, there does seem to be a trend indicating a positive correlation. The study is continuing and additional data may provide an answer to this question. The RIA has been shown to be an effective and accurate method of determining IgG antibody to specific malarial antigen in serum drawn under field conditions.

**REFERENCES:**

1. Stutz, D.R., McAlister, R.O., and Diggs, C.L.: Estimation of Anti-Malarial Antibody by Radioimmunoassay. *J. Parasitol.* In press. 1974.
2. Annual Report, SEATO Medical Research Laboratory, April 1973.
3. Rodbard, D.: Statistical Aspects of Radioimmunoassays: In Odell, W.D. and Daughaday, W.H. (eds), *Principles of Competitive Binding Assays.* J.B. Lipincott Co., 1971.