

Evaluation and Refinement of a Radioimmunoassay for the Quantitative Estimation of Malarial Antibody

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OBJECTIVE: To refine, field test and evaluate a radioimmunoassay for quantitative estimation of specific malarial antibody.

DESCRIPTION: A radioimmunoassay was developed at WRAIR for the purpose of quantitatively estimating specific malarial antibodies in human serum. Specific antibody is measured by inhibition of binding between monospecific antiglobulin and radiiodinated purified immunoglobulin. Preliminary experiments indicated that the method can be used to detect quantities of specific antibody as low as 0.05 $\mu\text{g/ml}$ and purified IgG in concentrations of 0.01 $\mu\text{g/ml}$. The development of the assay and tests for sensitivity and reliability were accomplished using pools of sera obtained from individuals living in areas of high endemicity. The assay proved to be reliable and the data easily reproducible.

The insolubilization method chosen for this assay was that of sensitizing sheep red blood cells (SRBC) with the antigen and using a prescribed number of cells per tube as the standardized antigen. Three major problems are inherent in this system: (1) the actual amount of antigen bound to the cells is difficult to determine; (2) different antigen preparations are difficult to standardize; and (3) all sera have to be adsorbed onto SRBC prior to being used in the assay. Due to these problems, a new means of insolubilizing the antigen was sought. Catt and Trager (1967) found that immunoglobulins would bind to polymers of plastics which are used in making disposable culture tubes. Further experimentation indicated that other proteins would also bind. The malaria antigen used in the radioimmunoassay is basically protein in nature, therefore this technique was selected for evaluation as a better means of antigen insolubilization.

PROGRESS: The antigen preparation is radiolabeled with ¹²⁵I and varying concentrations are used to coat 12 X 75 mm polypropylene tubes. The tubes are positioned so that a prescribed amount of fluid will cover the same surface area of the tube each time. Care is taken not to splash any antigen on the side of the tube. A coating period of 2 hours at room temperature has been shown to be the most satisfactory of those tried. No significant increase in antigen binding is observed with longer periods of time. A concentration of 10 $\mu\text{g/ml}$ was found to provide optimal coating. Approximately 11% of the radiolabeled antigen is bound to the tube at this concentration. After the antigen solution is removed, the tube is washed 5 times and filled with a 3% human serum albumin—phosphate buffered saline solution to fill the remainder of the binding sites and to remove the loosely bound antigen.

Repeated experiments with this tube coating process have shown that when 0.5 ml of a 10 $\mu\text{g/ml}$ coating solution is used, 0.285 μg (± 0.023) is bound to the tube. A pH of 7.5 is optimal as opposed to that found by Catt and Trager (pH 9.5—10.0). Experiments conducted at 4°, 22° and 37°C indicate that 22°C (room temperature) is optimal for binding. Coated tubes have been stored at 4°C for as long as 7 days and no loss of antigenicity was observed. Experiments are presently being conducted to determine how long these coated tubes can be stored at 4° and -70°C without loss of antigenicity.

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The protocol for the radioimmunoassay requires a standard curve to be established for each assay conducted. In order to have comparable conditions existing in both the standard and the antigen tubes, a series of experiments were conducted on the binding capability of purified human IgG. These experiments indicated that the same optimal conditions existed for the IgG as for the malaria antigen. It is possible to bind approximately 50% of varying concentrations (0.25 to 4.0 $\mu\text{g/ml}$) of ^{125}I -IgG to the tubes and the repeatability is within a 5% error.

Preliminary experiments with the pooled immune serum indicate that results are comparable to those obtained with the SRBC carrier. Initial experiments with individual serum samples collected in Thailand indicate that the tube-bound malaria antigen will detect specific antibody in relatively low concentrations ($< 5 \mu\text{g/ml}$). Future experiments will be designed to increase the sensitivity.

SUMMARY: A radioimmunoassay has been modified as to the method of insolubilizing the antigen. Preliminary experiments indicate that data derived after this modification is comparable to that with the original assay.

REFERENCE:

1. Catt, K. and Trager, G.: Solid-phase immunoassay in antibody coated tubes. *Science* 158: 1570, 1967.