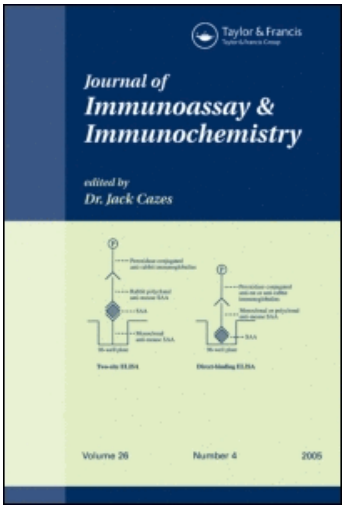

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Theoretical Analysis of the Parr Antibody Assay with a Computer Model: Importance of Antigen Concentration and Antibody Affinity

William R. Griswold[a]; Dennis P. Nelson[a]
[a] University of California at San Diego and the Naval Health Research Center, San Diego, California

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THEORETICAL ANALYSIS OF THE FARR ANTIBODY ASSAY WITH A COMPUTER MODEL: IMPORTANCE OF ANTIGEN CONCENTRATION AND ANTIBODY AFFINITY

William R. Griswold and Dennis P. Nelson
University of California at San Diego and the
Naval Health Research Center, San Diego, California

ABSTRACT

The Farr assay for antibody was analyzed theoretically in order to determine the maximum sensitivity of the assay, the quantitative relationship between actual antibody concentration and antibody estimates by the Farr technique, and the relationship between antibody affinity and the Farr avidity index. Analysis shows that sensitivity is limited by the antigen concentration when large amounts of antigen are used in the assay. Sensitivity at low antigen concentrations is maximal and varies with the affinity of the antibody studied. Antibody titers obtained by the Farr technique using low antigen concentrations vary with antibody affinity as well as antibody concentration. Titers measured at high antigen concentration are less affected by affinity and correlate better with antibody concentration. Antibody measurements expressed as Antigen Binding Capacity reflect antibody concentration only when the antigen concentration used in the assay exceeds a value equal to ten times the reciprocal of the antibody affinity constant. The Farr avidity index correlates with antibody affinity over a narrow affinity range. Different ranges of affinity can be examined by changing the antigen concentrations.
(KEY WORDS: ANTIBODY MEASUREMENT, ANTIBODY AFFINITY, FARR TECHNIQUE)

INTRODUCTION

Farr originally described a quantitative assay to study the primary interaction of antibody with antigen (1). Since the antigen is radiolabeled, total antigen is easily

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determined. Antigen bound to antibody is measured by precipitation in half saturated ammonium sulfate. The assay requires that free antigen remains soluble in this solution. This important technique has been used to detect antibody against a variety of antigens including bovine serum albumin (BSA) (1), DNA (2), hemoglobin (3), and streptococcal M protein (4). A review of the literature shows that antibody measurements have been made at a variety of antigen concentrations. Anti-DNA antibody is usually assayed at antigen concentrations between 10^{-8} and 10^{-9}M (6,7). Antibody to BSA has been determined at antigen concentrations varying over a wide range from 10^{-5} to 10^{-9}M (1,5,8).

Although the Farr technique has received widespread application, many theoretical aspects of the method have not been described in the literature. This study was undertaken to analyze the Farr antibody assay from a theoretical standpoint. Several features of the assay were examined: 1. the sensitivity of the assay; 2. the relationship between antibody measurements obtained by the Farr assay and actual antibody concentration; and 3. the relationship between the the Farr avidity index and antibody affinity. This analysis provides a better understanding of the conditions which should be used in the assay and the significance of antibody measurements obtained with this technique.

MATERIALS AND METHODS

Antigen and antibody were assumed to interact according to the classical binding reaction to form a complex.

$$\text{Antigen} + \text{Antibody} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Antigen-Antibody Complex}$$

The antigen-antibody reaction is governed by the thermodynamic equilibrium equations as follows:

$$k_1 / k_2 = K_a = (AgAb) / (Ag) * (Ab)$$

where k_1 and k_2 are the forward and reverse rate constants respectively, K_a is the antibody affinity constant, Ag is free antigen, Ab is free antibody, and AgAb is antigen-antibody complex. Parentheses indicate concentrations. This equation can be solved, as shown in the Appendix, for fraction antigen bound in terms of total antibody concentration (TAb), total antigen concentration (TAg) and antibody affinity (K_a).
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Antibody Titer

To simulate determination of antibody titer by the Farr assay, a fixed antigen concentration and antibody affinity were chosen. The initial concentration of antibody in undiluted serum was assumed to be 10^{-5}M. A series of antibody concentrations based on progressive dilutions starting at 10^{-5}M was computer generated. The concentration of bound antigen and the percent antigen bound to antibody was calculated for each antigen concentration. These calculations were repeated using antigen concentrations between 10^{-5} and 10^{-10}M and affinity values between 10^6 and 10^{10}M^{-1}. The highest dilution of antibody which bound at least 10% of the antigen was selected as the titer.

Antigen Binding Capacity

Antigen binding capacity or ABC values are found experimentally by determining the dilution of antibody which

binds a specified fraction of the antigen. Farr originally chose an endpoint of 33% net antigen bound (1); other investigators have used 10% or less as the endpoint (9). ABC is calculated by multiplying the fraction antigen bound by the concentration of antigen in the test system. The concentration of bound antigen is then multiplied by the reciprocal of the antiserum dilution to standardize the ABC per ml of serum. This can be expressed mathematically as follows:

$$ABC = F * (TAg) * 1/D$$

where F is the fraction antigen bound, (TAg) is the total antigen concentration and D is the antibody dilution.

In the computer simulation ABC values were calculated assuming that the initial antibody concentration was 10^{-5}M. Ten percent net antigen bound was chosen as the endpoint. ABC values are expressed as a percent of the reference antibody concentration, 10^{-5}M.

Relative Avidity Index

The relative avidity index was described by Farr in the original paper on the ammonium sulfate assay (1). This index was taken as the ratio between the ABC values obtained at two different antigen concentrations multiplied by 100. The antigen concentrations Farr used were roughly 10^{-9} and 10^{-8}M for BSA.

In the computer simulation the relative avidity index was calculated using the equation:

$$\text{Relative Avidity Index} = ABC_1/ABC_2 \times 100$$

where ABC_1 is the ABC at 10^{-9}M antigen concentration and ABC_2 is the ABC at 10^{-8}M antigen concentration. ABC values were calculated as described in the previous section. The avidity index was also calculated using ABC values at two other combinations of antigen concentrations, 10^{-8} versus 10^{-7}M, and 10^{-7} versus 10^{-6}M.

RESULTS

Sensitivity of the Farr Antibody Assay

The sensitivity or minimum detectable concentration of antibody is shown in Figure 1. At high antigen concentrations the sensitivity is a function of the amount of antigen. At low antigen concentrations sensitivity reaches maximum values which are independent of antigen concentration. The maximum sensitivity varies with antibody affinity. In general the higher the affinity the more sensitive the assay. In Figure 1 the minimum antibody concentration detectable equals the reciprocal of the affinity constant multiplied by 0.1.

Relationship between antibody measurements, antibody affinity, and antibody concentration

Measurement of antibody titer was simulated with the computer model. The titer was defined as the highest dilution of antibody capable of binding at least 10% of the

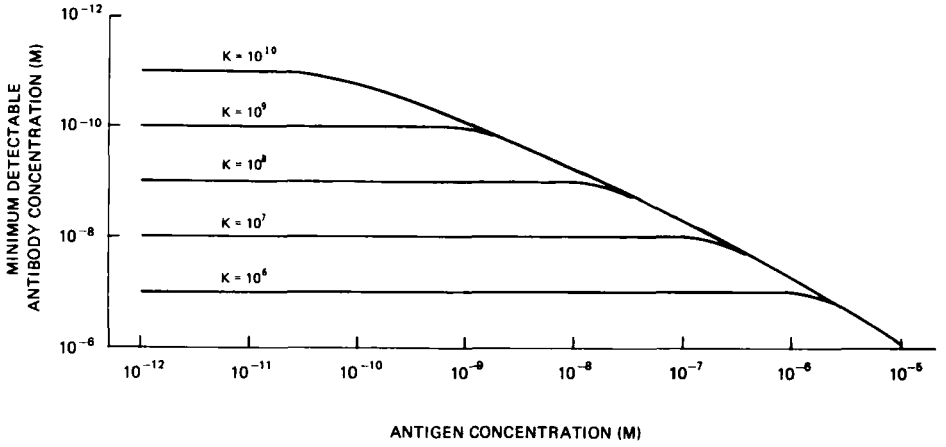

FIGURE 1 The theoretical relationship between sensitivity of the Farr assay, antibody binding constant, and antigen concentration.

antigen. Initial pre-dilution antibody concentration was set at 10^{-5}M and was identical for all simulation studies. The results are shown in Figure 2. Except at the highest antigen concentrations, such as 10^{-5}M, titer varies with affinity. At the antigen concentration used most commonly in the Farr assay, 10^{-9}M, the antibody titer varies more than one thousandfold between affinity values of 10^{6} and $10^{10}{}_{M}{}^{-1}$.

The percent antigen bound to antibody also varies markedly with antibody affinity as shown in Figure 3. Total antigen and total antibody were held constant during this analysis. When higher concentrations of antigen are used the variation of antigen bound with affinity is less pronounced.

Measurements of antibody expressed as Antigen Binding Capacity (ABC) also vary with antibody affinity (Figure 4).

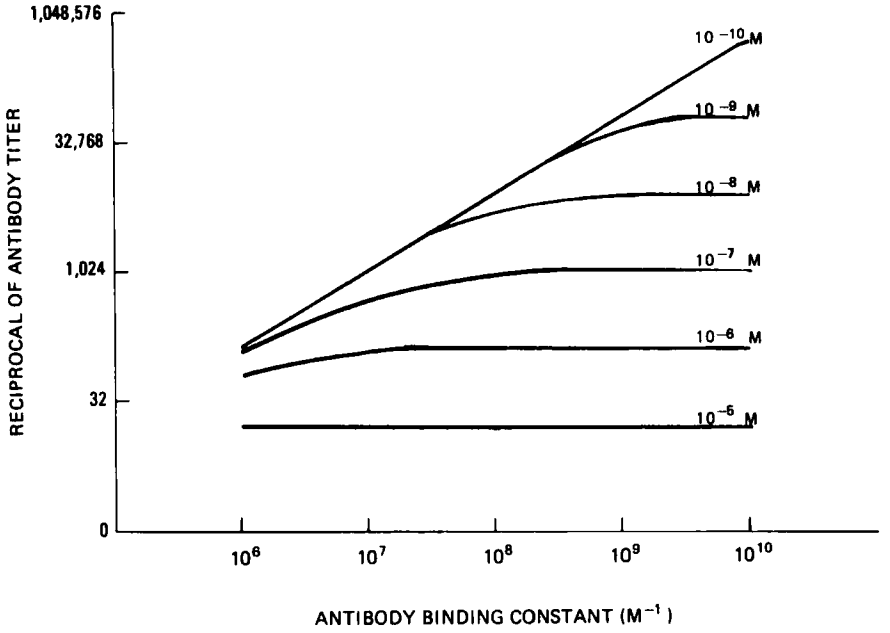

FIGURE 2 The theoretical relationship between the antibody constant and antibody titer in the Farr assay. Pre-dilution antibody concentration was 10^{-5}M. Antigen concentration (M) for each curve is shown on the graph.

The ABC values accurately reflect the actual antibody concentration when the antibody affinity is high, but underestimate antibody concentration when affinity is low. As a general rule, ABC values accurately reflect the antibody concentration only when the antigen concentration is greater than ten times the reciprocal of the antibody binding constant.

Relative Avidity Index

This parameter was defined by Farr as the ratio of ABC values obtained at two different antigen concentrations (1).

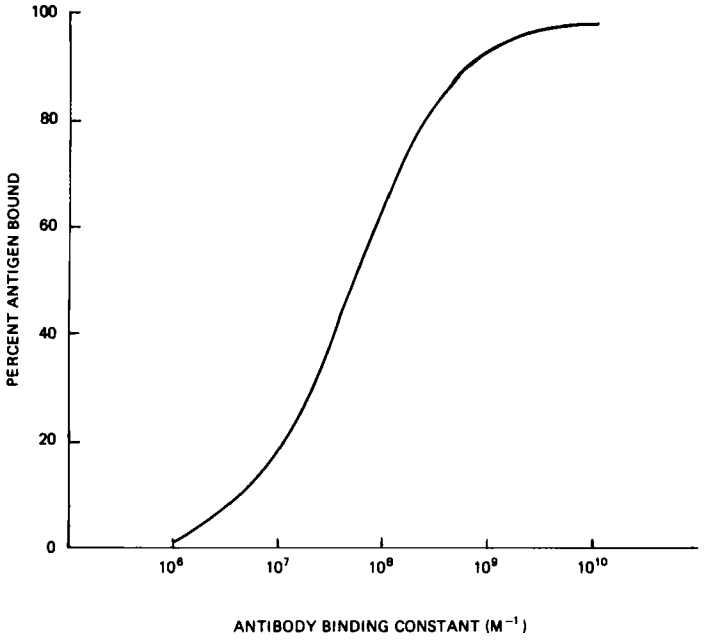

FIGURE 3 The theoretical relationship between the antibody binding constant and percent antigen bound in the Farr assay. Total antigen concentration is 10^{-9} M. Total antibody concentration is 1.95×10^{-8} M.

Theoretical analysis shows that the avidity index correlates with the affinity of antibody (Figure 5). The range over which the index correlates with affinity is determined by the antigen concentration. With higher antigen concentrations the range of antibody affinity covered by the avidity index shifts to lower values.

DISCUSSION

The sensitivity of the Farr technique for antibody detection varies with the concentration of antigen used in the assay and the affinity of antibody being studied. At

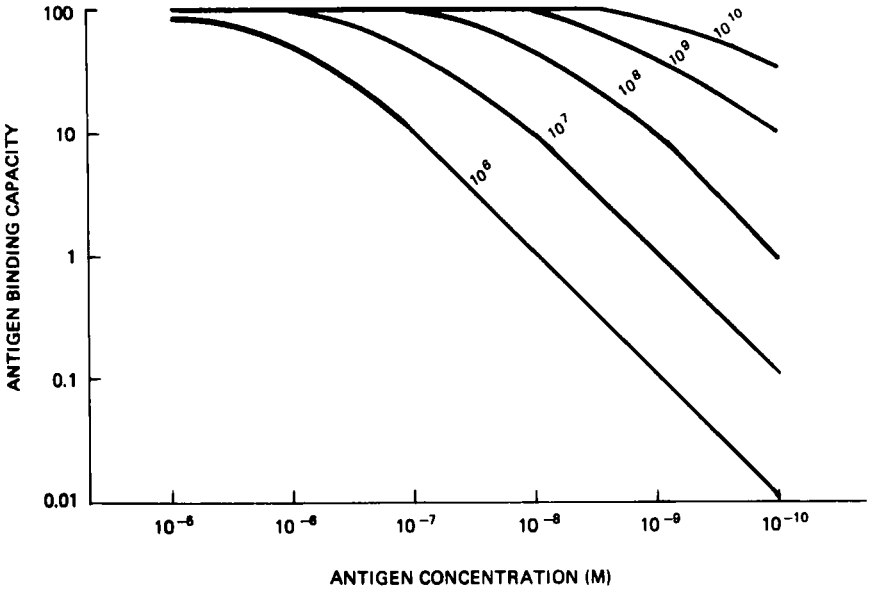

FIGURE 4 The theoretical relationship between the antibody binding constant and Antigen Binding Capacity (ABC). ABC values are expressed as percent of actual antibody concentration. The curves are labeled with the affinity constants in M^{-1}.

high antigen concentration the sensitivity varies with the antigen concentration and is independent of antibody affinity. When the antigen concentration is low, the sensitivity reaches a maximum which varies with the antibody affinity. Sensitivity could also be improved by lowering the minimum detectable percent antigen bound. The calculations in this study were based on the assumption that ten percent net antigen bound was the minimum fraction that could be measured accurately. Although lower endpoints are theoretically possible, the error due to variability in non-specific antigen precipitation becomes large as the

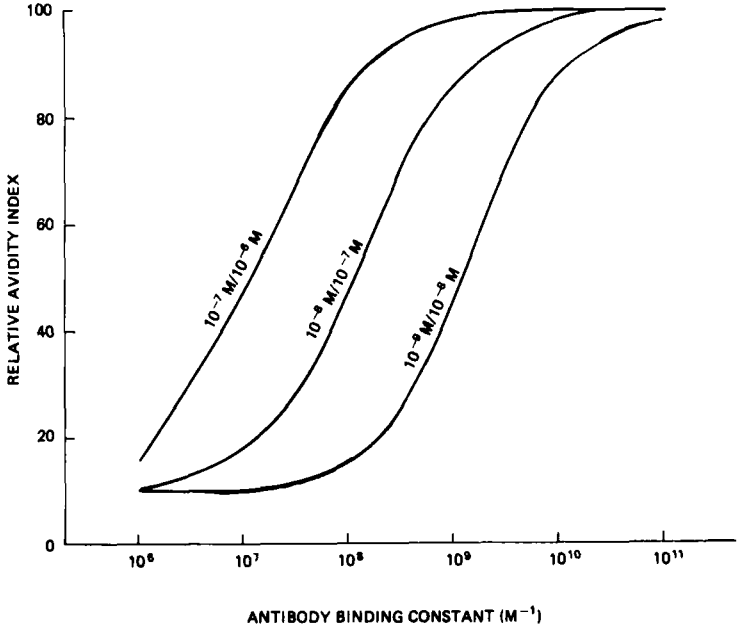

FIGURE 5 The theoretical relationship between the antibody binding constant and the relative avidity index of Farr. The curves are labeled with the pair of antigen concentrations used for the ABC_1 and ABC_2 measurements.

percent antigen bound decreases. Thus ten percent is a reasonable choice for the endpoint unless special techniques are used to control the error due to non-specific antigen precipitation.

Antibody measurements obtained with the Farr assay are often expressed in arbitrary units such as titer, percent antigen bound, or ABC values. Most antibody assays are performed at low antigen concentrations. Under these conditions the antibody measurements will vary with antibody affinity as well as antibody concentration. Thus a low antibody titer may just as well result from low antibody

affinity as low antibody concentration. The effect of affinity on antibody measurements can be minimized by using higher concentrations of antigen. When more antigen is used the titers are influenced less by affinity and correlate better with antibody concentration. In practical terms antibody titers measured with low antigen concentrations have proven to be clinically significant since antibody affinity is likely to be as important clinically as antibody concentration.

When homogeneous antibody populations, such as monoclonal antibodies, are studied with the Farr technique, the concentration of antibody binding sites can be estimated by determining the antigen binding capacity with an antigen concentration equal to or greater than ten times the reciprocal of the antibody affinity constant. Antisera obtained from immunized animals are known to contain a spectrum of antibodies with different affinities (9). When antibody is heterogeneous with respect to affinity the Farr assay reflects only a subpopulation of the antibodies present. The range of the subpopulation is determined by the antigen concentration. The assay will provide an estimate of the concentration of antibodies having an affinity greater than or equal to ten times the reciprocal of the antigen concentration used in the assay. Antibodies with affinity equal to the reciprocal of the antigen concentration will be partially measured. Only a small fraction of antibodies with a lower affinity will be detected.

Werblin and Siskind (9) have shown that most antisera obtained from immunized animals contain significant amounts of low affinity antibody. In these antisera measurement of the total antibody concentration will require a concentration of antigen of 10^{-5}M or more. This phenomenon was also shown by Osler et.al. (8) in studies of antisera obtained from rabbits hyperimmunized with BSA. These investigators found that antigen concentrations of 10^{-5}M were required to saturate all of the antibody binding sites, confirming that even hyperimmune antisera contain significant amounts of low affinity antibody.

This computer model was developed for univalent antigens. If antigen is multivalent, more than one antibody may attach to the antigen. The Farr technique cannot, however, distinguish between antigen with one or multiple antibody molecules attached. When antigen is multivalent the Farr technique may underestimate the concentrations of antibody. This effect can be minimized by using antigen excess conditions. When a large excess of antigen is present the likelihood that any one antigen molecule will bind more than one antibody molecule is small. Thus in antigen excess even multivalent antigens behave as if they were univalent.

Theoretical analysis of the Farr avidity index shows that this parameter correlates with antibody affinity. The avidity index can be used to estimate an approximate antibody binding constant from Figure 5 of this report.

The Farr assay is an important technique for the study of antibody. An understanding of the theoretical behavior

of the assay provides a better foundation for interpreting the results obtained by this valuable immunoassay.

ACKNOWLEDGMENTS

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REFERENCES

1. Farr, R.S. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. J. Infec. Dis. 1958; 103: 239-262.

2. Pincus, T., Schur, P.H., Rose, J.A., Decker, J.L., and Tular, I.V. Measurement of serum DNA binding activity in Systemic Lupus Erythematosus. New Eng. J. Med. 1969; 281: 701-705.

3. Garver, F.A., and Talmage, D.W. The inhibitor of the primary interaction between ^{125}I labeled human hemoglobin and rabbit anti-hemoglobin sera: A sensitive radioimmunoassay technique. 1975; 7: 271-282.

4. Grey, H. Studies on the binding between Streptococcal M protein and antibody. J. Exp. Med. 1962; 114: 161-170.

5. Minden, P., Anthony, B.F., and Farr, R.S. A comparison of seven procedures to detect the primary binding of antigen by antibody. J. Immunol. 1969; 102: 832-841.

6. Aarden, L.A., Lakmaker, F., and Feltkamp, T.E.W. Immunology of DNA. II. The effect of size and structure of the antigen on the Farr assay. J. Immunol. Meth. 1976; 10: 39-48.

7. Geisert, M., Heicke, B., Metzmann, E. and Zahm, R.K. Influence of molecular weight of DNA on the determination of anti-DNA antibodies in systemic lupus erythematosus sera by radioimmunoassay. Nucleic Acids Res. 1975; 2: 521-535.

8. Osler, A.G., Mulligan, J.J. and Rodriguez, E. Weight estimates of rabbit anti-human serum albumin based on antigen-binding capacity. J. Immunol. 1966; 96: 324-333.

9. Werblin, T.P. and Siskind, G.W. Distribution of antibody affinities: Technique of measurement. Immunochem. 1972; 9: 987-1011.

APPENDIX

TAg	Total Antigen Concentration
TAb	Total Antibody Concentration
Ag	Free Antigen Concentration
Ab	Free Antibody Concentration
AgAb	Bound Antigen-Antibody Complex Concentration

From equilibrium considerations

$$K = AgAb/(Ag*Ab)$$

Rearranging

$$AgAb = K*Ag*Ab \qquad (1)$$

Also from mass balance

$$TAb = Ab + AgAb \qquad (2)$$

$$TAg = Ag + AgAb \qquad (3)$$

Substituting

$$TAg = Ag + K*Ag*Ab$$

$$TAg = Ag*(1 + K*Ab)$$

Rearranging

$$Ag = TAg/(1 + K*Ab) \qquad (4)$$

Similarly

$$TAb = Ab + K*Ab*Ag$$

Using 4 to substitute for Ag

$$TAb = Ab+(K*Ab*TAg)/(1+K*Ab) \qquad (5)$$

Using equations (2) and (5)

$$AgAb = TAb - Ab$$

or

$$AgAb=(K*Ab*TAg)/(1+K*Ab)$$

To solve for AgAb knowing K and TAg, Ab must be determined

Solving (5) in terms of Ab

$$K*Ab^2 + (1+K*(TAg-TAb))*Ab - TAb = 0$$

Thus Ab is the positive root of a quadratic equation. Once Ab is known the concentration of all other species can be calculated.