Interrelations of the Various Mathematical Approaches to Radioimmunoassay

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Equations used for calculation of radioimmunoassay data are derived and compared. We show that the equation developed by Fernandez and Loeb is the most general of these. Those of Ekins et al., Hales and Randle, and Rodbard et al. are all derived here from this basic equation by neglecting certain of its terms. The logit-log plot of Rodbard et al. is a logarithmic transformation of the linear Hales–Randle equation. The basic equation, derived here solely from the mass-action law, approaches linearity under certain conditions without complex mathematical transformations of analyte concentrations or observable parameters. Applications are also presented.

With each new technique the analyst has to work out methods to calculate analyte concentrations from his experimental data. The direct approach is simply to plot some values for the chosen variable vs the corresponding concentrations to obtain a calibration curve. To be useful quantitatively, the calibration curve must be reproducible under specified conditions. The unknown value for a particular sample is obtained by interpolation on the graph.

Because the form of a curve cannot be determined unambiguously from the necessarily few standard points, an exact curve cannot be obtained in this empirical way. The lower the precision of the experimental results, the more uncertain is the geometry of the curve. However, if a theoretically sound equation is available that can relate all the parameters involved, the analytical system is completely described. Consequently, conditions adequate for a given test can be selected, causes of problems determined, and problems corrected. It should be possible with appropriate mathematical treatment to express the theoretical equation in such a way that it gives a linear relationship between the directly measured variable and the concentration of analyte. Use of this linear function simplifies selection of the line that best fits the standard points and facilitates interpolation of the unknown values.

Since the advent of radioimmunoassay 20 years ago, many attempts have been made to correlate the concentrations of the analytes with the corresponding counts of free or bound radioactivity (1–5). Some have used empirical approaches (1, 4), others have attempted theoretical derivations of the formula (2, 3, 5). For proper use of them it is important to understand how the different approaches are interrelated and how rigorous the corresponding equations are.

Here, we describe the interrelationships of these various approaches in a more general way and show that there is an equation common to all of them, but more comprehensive. This equation has been proposed previously (5), but we will show here it may be derived solely from the mass-action law. Because of its importance in the unification of the different mathematical treatments of RIA systems, its derivation will be shown in detail.

Derivation of the Basic Equation

When an equilibrium is reached in a radioimmunoassay system between the ligand P and the binder Q, it can be described by the expression P + Q ≡ PQ, where PQ is the ligand–binder complex. Likewise, with a radioactive ligand P* we have P* + Q ≡ P*Q. When all these substances are present simultaneously, the equilibrium can be expressed by

\[ P^* + Q \rightleftharpoons P^*Q \]

If the bound fraction (PQ + P*Q) is separated from the free ligand (P + P*) and its radioactivity measured, it can be seen that radioactivity will be maximum when the added P is zero. Likewise, as P increases the free Q is diminished and P*Q and its corresponding bound radioactivity decreases. Expression 1 provides a good qualitative description of a radioimmunoassay system and conveys the basic principle involved. However, for a quantitative description, a mathematical equation including all the pertinent parameters is required. The equilibrium represented by expression 1 is governed by the mass-action law first presented by Guldberg and Waage (6). Because there are two ligands involved, P and P*, the mass-action law defines two equilibrium constants

\[ K = \frac{[PQ]}{[P][Q]} \quad (2) \]

and their reciprocals

\[ K^{-1} = \frac{[P]}{[Q]} \quad (3) \]  

\[ K^{-1} = \frac{[P^*Q]}{[P^*][Q]} \quad (3a) \]

Quantities in brackets are the molar concentrations of the respective constituents. In thermodynamic expressions, activities would be used in place of molar concentrations, but with very dilute solutions and limited ranges of concentration the loss of accuracy with use of molar concentrations is negligible.

Solving for [Q] in equations 3 and 3a, and rearranging, we obtain:

\[ \frac{K^{-1}}{K^*} \cdot [PQ] = [P^*Q] \]

\[ \frac{[P^*]}{[P]} = \frac{[PQ]}{[P^*][Q]} \]
If we restrict our treatment to the case in which $K^{-1} = K^{*-1}$ then on adding 1 to each side and reducing to a common denominator we obtain:

$$\frac{[P] + [PQ]}{[P]} = \frac{[P*] + [P*Q]}{[P*]} \quad (4)$$

Performing the same operations on the reciprocals, we obtain:

$$\frac{[P] + [PQ]}{[PQ]} = \frac{[P*] + [P*Q]}{[P*Q]} \quad (5)$$

These concentration terms cannot be pre-set or easily measured, so we will need to express them as functions of other readily measured parameters. Thus, taking

$$p = [P] + [PQ] = \text{total concentration of unlabeled ligand}$$

$$p* = [P*] + [P*Q] = \text{total concentration of labeled ligand}$$

and substituting these values in equations 4 and 5, we obtain:

$$\frac{p}{[P]} = \frac{p*}{[P*]} \quad (6) \quad \text{and} \quad \frac{p}{[PQ]} = \frac{p*}{[P*Q]} \quad (7)$$

Equations 6 and 7 are independent of the equilibrium constant and express the distributions of radiolabeled substances and their unlabeled counterparts when they have the same equilibrium constant. Rearranging equations 3 and 3a, using the same $K^{-1}$ for both, taking their sum, and solving for $K^{-1}$, we obtain:

$$K^{-1} = \frac{[P] + [P*]}{[PQ] + [P*Q]} \quad (8)$$

Defining

$$V = \text{volume of undiluted binder added}$$

$$q = \text{concentration of binding sites in the undiluted binder reagent}$$

$$W = \text{total volume of incubation mixture}$$

$$q = [PQ] + [P*Q] + [Q] = \text{total concentration of binder in the incubation mixture}$$

then the total concentration of binder is

$$\frac{qV}{W} = [PQ] + [P*Q] + [Q] \quad (9)$$

Solving for $[Q]$:

$$[Q] = \frac{qV}{W} - [PQ] - [P*Q]$$

Replacing this equivalent of $[Q]$ in equation 8:

$$K^{-1} = \frac{[P] + [P*]}{[PQ] + [P*Q]} \left( \frac{qV}{K^{-1}W} - [PQ] - [P*Q] \right) \quad (10)$$

Rearranging:

$$\frac{1}{[P] + [P*]} = \frac{1}{[PQ] + [P*Q]} \left( \frac{qV}{K^{-1}W} - [PQ] - [P*Q] \right) \quad (11)$$

Multiplying and dividing the right side by $[PQ] + [P*Q]$ gives:

$$\frac{1}{[P] + [P*]} = \frac{qV}{K^{-1}W} \cdot \frac{1}{[PQ] + [P*Q]} - \frac{1}{K^{-1}} \quad (12)$$

Substituting $[P]$ and $[PQ]$ from equations 6 and 7 into equation 11, we obtain:

$$\frac{p}{p*} \left( \frac{1}{[P*] + [P*Q]} \right) = \frac{qV}{K^{-1}W} \cdot \frac{1 - \frac{1}{K^{-1}}}{[P*Q] + [P*Q]} \quad (13)$$

Taking out the common factors $[P*]$ and $[P*Q]$ and then multiplying each side by

$$\left( \frac{p}{p*} + 1 \right) p* \quad \text{gives:}$$

$$\frac{p}{p*} = \frac{qV}{K^{-1}W} \cdot \frac{p*}{[P*Q]} - \frac{p + p*}{K^{-1}} \quad (13)$$

Let $p = p_r + p_x$ total unlabeled ligand concentration in the incubation mixture, $p_r$ = concentration of unlabeled ligand in the incubation mixture contributed by the labeled reagent, and $p_x$ = concentration of unlabeled ligand in the incubation mixture contributed by the standard or unknown. On substituting $p_r + p_x$ for $p$ in equation 13 and rearranging, we obtain:

$$\frac{p*}{[P*Q]} = \frac{K^{-1}W}{qV} \cdot \frac{p*}{[P*Q]} + \frac{W}{qV} (p_r + p*) + \frac{W}{qV} p_x \quad (14)$$

Let $T = \text{total counts of radioactivity in the incubation mixture}$, $B = \text{counts of radioactivity in the bound fraction}$, and $F = \text{counts of radioactivity in the free ligand fraction}$. Then

$$\frac{p*}{[P*Q]} = \frac{T}{B} \quad \text{and} \quad \frac{p*}{[P*]} = \frac{T}{F} \quad (15)$$

Substituting these expressions in equation 14

$$\frac{T}{B} = \frac{K^{-1}W}{qV} \cdot \frac{T}{F} + \frac{W}{qV} (p_r + p*) + \frac{W}{qV} p_x \quad (15)$$

Defining

$p_r = \text{concentration of unlabeled ligand in the undiluted label reagent}$

$p* = \text{concentration of labeled ligand in the undiluted label reagent}$

$U = \text{volume of undiluted labeled ligand reagent added to the incubation mixture}$, then

$$p_r = \frac{\hat{p}_r U}{W} ; \quad p* = \frac{\hat{p}_* U}{W} \quad \text{and} \quad p_r + p* = \frac{(p_r + p*) U}{W}$$

Substituting this last expression in equation 15

$$\frac{T}{B} = \frac{K^{-1}W}{qV} \cdot \frac{T}{F} + \frac{W}{qV} \left( \frac{p_r + p*}{W} + \frac{W}{qV} p_x \right) \quad (16)$$

Defining

$$y = \frac{T}{B} \quad \text{and since} \quad F = T - B,$$

then

$$\frac{T}{F} = \frac{T}{T - B} = \frac{T/B}{(T/B) - 1} = \frac{y}{y - 1} \quad (16)$$

Substituting these $y$ terms in equation 16
\[
y = \frac{K^{-1} W}{q V} \left( \frac{y}{y - 1} \right) + \frac{(p_r + p*) U}{q V} + \frac{W}{q V} p_r \tag{17}
\]

Let \( P_x = W p_x \) = mass of ligand in the incubation mixture contributed by the standard or unknown, we finally have

\[
y = \frac{(p_r + p*) U}{q V} + \frac{K^{-1} W}{q V} \left( \frac{y}{y - 1} \right) + \frac{1}{q V} p_r \tag{18}
\]

This is the basic theoretical equation governing an ideal radioimmunoassay system. It is applicable to systems at equilibrium having a homogeneous antibody or binder with a single equilibrium constant, \( K^{-1} \), both for labeled and unlabeled ligand and for all binding sites. \( K^{-1} \) is defined in equation 3 as a ratio of molar concentrations, so the units of \( K^{-1} \) are moles per liter. In the application of equation 18 it is more convenient to use milliliters instead of liters; weight units in \( \mu g, \text{ng}, \text{etc.} \), instead of moles; and concentrations in weight/mL instead of moles/liter. Consequently, \( K^{-1} \) will have the same units of weight/mL. When these units are adopted, the binder concentration must be expressed in terms of binding capacity for the ligand in the same units of weight/mL. These considerations are equally applicable to the other equations discussed here. The advantages of equation 18, as discussed previously (5), are linearity when the variation of the second term is negligible as compared with the sum of the first and second terms, ease of calculation of the fundamental parameters, and the simple experimental control of the system that is achieved by selection of volumes \( U, V, \) and \( W \), which together govern the slope, intercept, and linearity.  

Interrelationships of the Commonly Used Mathematical Approaches

The equations of Ekins et al. In 1968 Ekins et al. (2) published the derivations of two equations, both based on the same restrictive premises as above. One is a function of \( F/B \), the other of \( B/F \). In their derivation they use \( p \) to represent total ligand, but in their experimental plots \( p \) represents, as must all standard plots, the concentration of added standard. They noted this when, in plotting theoretical values (their Figure 5a) and experimental values (their Figure 5b), they stated that, "The two illustrations differ slightly in that the concentration of tracer hormone present in the system is disregarded in Fig. 5b." Whenever the field of application of the equation of Ekins et al. is to be extended to the description of calibration curves, the concentration of tracer ligand in the system must be disregarded. By doing so the value of \( p \) is restricted to the concentration of added standard (or unknown) as the independent variable. Therefore we can reconcile the equations of Ekins et al. in this more restricted form with the basic equation 18 if the first term of equation 18 is omitted. By doing so we have

\[
y = \frac{K^{-1} W}{q V} \left( \frac{y}{y - 1} \right) + \frac{1}{q V} P_x \tag{19}
\]

Dividing numerator and denominator by the total volume \( W \)

\[
y = \frac{K^{-1}}{q V/W} \left( \frac{y}{y - 1} \right) + \frac{P_x/W}{q V/W} \tag{20}
\]

But the total concentration of binder is \( q = q V/W \) and

the total concentration of standard is

\[
p = \frac{p_x W}{W}, \text{ so} \quad y = \frac{K^{-1}}{q} \left( \frac{y}{y - 1} \right) + \frac{p}{q} \tag{21}
\]

Multiplying both sides by \( \left( y - \frac{1}{y} \right) q \) and rearranging, we obtain:

\[
\frac{1}{y - 1} = \frac{q}{p \left( y - \frac{1}{y} \right) + K^{-1}}
\]

Since

\[
\frac{1}{y - 1} = \frac{1}{T} = \frac{B}{B - 1} = \frac{B}{F}
\]

and

\[
\frac{y - 1}{y} = \frac{T}{B - 1} = \frac{T - B}{F} = \frac{F}{F + B} = \frac{1}{1 + \frac{B}{F}}
\]

Substituting these \( B/F \) expressions in equation 21 gives

\[
\frac{B}{F} = \frac{q}{1 + \frac{1}{K} + \frac{1}{K}}
\]

Using the notation of Ekins et al., \( R_{\text{ef}} \) equals \( B/F \)

\[
R_{\text{ef}} = \frac{p}{1 + R_{\text{ef}}} + \frac{1}{K} \tag{22}
\]

The reciprocal of equation 22 gives directly the second equation of Ekins

\[
R_{\text{ef}} = \frac{p}{q \left( 1 + \frac{1}{R_{\text{ef}}} \right)} + \frac{1}{Kq} \tag{23}
\]

These equations of Ekins et al., 22 and 23, with the independent variable \( p \) representing the concentration of added standard are therefore reconcilable with an approximate form of the basic equation 18.

The equation of Hales and Randle. In 1963, Hales and Randle proposed (3) a simple linear equation, which does not include the equilibrium constant \( K \). This then is an approximate equation applicable to the case in which \( K^{-1} \) is so small that its influence can be neglected. We show here that by omitting the second term of the basic equation 18 the equation of Hales and Randle may be derived from it. Without the second term, equation 18 takes the form

\[
\frac{1}{y - 1} = \frac{q}{p \left( y - \frac{1}{y} \right) + K^{-1}}
\]

2 A theoretical equation expressing \( B/T \) as a quadratic function of the concentration of added standard is presented in Meinert CL, McHugh RB. The biometry of an isotope displacement immunologic microassay (Math Biosci 2: 319, 1968). This function is compatible with equation 18, but is not linear.
\[ y = \frac{(p_r + p^*)U}{qV} + \frac{1}{qV} P_x \] (24)

When the standard = 0,
\[ P_x = 0, y = y_0, \text{ and } B = B_0. \]

Then
\[ y_0 = \frac{(p_r + p^*)U}{qV} \] (25)

Dividing equation 24 by equation 25 gives
\[ \frac{y}{y_0} = 1 + \frac{1}{(p_r + p^*)U} P_x \] (26)

Since \( \frac{y}{y_0} = \frac{T/B}{T/B_0} = \frac{B_0}{B} \)

substituting in equation 26
\[ \frac{B_0}{B} = \frac{1}{(p_r + p^*)U/W} P_x + 1 \] (27)

This equation is equivalent to the equation of Hales and Randle. It can be expressed in terms of concentration by dividing numerator and denominator of the first term on the right side by W.

Then, \( \frac{B_0}{B} = \frac{1}{(p_r + p^*)U/W} P_x/W + 1 \)

or, using the notation of Hales and Randle,
\[ i = P_x/W = \text{concentration of standard or unknown} \]
\[ i_0 = (p_r + p^*)U/W = \text{concentration of ligand contributed by the label reagent} \]
\[ C_i = B \]
\[ C_0 = B_0 \]
then we have
\[ \frac{C_0}{C_i} = \frac{i}{i_0} + 1 \] (28)

Equation 28 is the equation proposed by Hales and Randle. It is easily seen that \( C_0/C_i \) is a linear function of the concentration of standard i, that the intercept is 1 and the slope, \( 1/i_0 \), is the reciprocal of the concentration of ligand contributed by the label reagent. It can be seen that as a result of dividing equation 24 by equation 25, the factor \( qV \) is eliminated, making the subsequent expression independent of the quantity of ligand added.

The logit-log plot of Rodbard et al. In 1968, Rodbard et al. proposed that the logit-log plot be used for RIA calculations. Derived empirically, it generally gives linear plots (4).

We show here that the logit-log plot is a logarithmic expression of the equation of Hales and Randle, and therefore derivable from the basic equation 18 if the term containing \( K^{-1} \) is omitted. Beginning with equation 27,
\[ \frac{B_0}{B} = \frac{1}{(p_r + p^*)U} P_x + 1 \]

subtracting 1 from each side, reducing to a common denominator, taking reciprocals, dividing numerator and denominator of the left side by \( B_0 \), and finally taking logarithms

\[ \ln \frac{B_0/B}{1 - B_0/B} = \ln (p_r + p^*)U - \ln P_x \]

Since \( (p_r + p^*)U \) is constant, we define
\[ a = \ln (p_r + p^*)U \]

then
\[ \ln \frac{B_0/B}{1 - B_0/B} = a - \ln P_x \]

The term on the left is defined as the logit \( B/B_0 \)

then
\[ \logit \left( \frac{B}{B_0} \right) = a - \ln P_x \] (29)

Equation 29 represents the logit-log plot, and shows that the logit of \( B/B_0 \) is a linear function of the ln of the mass of standard \( P_x \). The slope of this function is \(-1\) and the intercept is \( "a" \), which equals \( \ln (p_r + p^*)U \), the ln of the mass of ligand contributed by the label reagent.

Since we have derived equation 29 from the basic equation 18, the logit plot is no longer empirical and the meaning of the intercept "a" is now clear.

All the relationships discussed above are condensed in Figure 1.

Application to Experimental Results

Data from a thyroxin (T4) analysis, run with use of the Pantex Immuno T4 Quick Kit, were used to plot five

\[ y = \frac{(p_r + p^*)U}{qV} + \frac{K^{-1}W}{qV} \left( \frac{y}{y-1} \right) + \frac{1}{qV} P_x \]

Basic equation of Fernandez and Loeb (1975)

Approximate form omitting the first term.

\[ y = \frac{K^{-1}W}{qV} \left( \frac{y}{y-1} \right) + \frac{1}{qV} P_x \]

Approxiante form omitting the second term.

\[ R_{lb} = \frac{p}{q(1 + 1/R_{lo})} + \frac{1}{Kq} \]

Hales and Randle equation (1963)

and

\[ R_{lo} = \frac{q}{1 + R_{lo}} + \frac{1}{K} \]

Ekings equations (1968)

\[ \logit \left( \frac{B}{B_0} \right) = a - \ln P_x \]

Logit-log plot of Rodbard (1968)

Fig. 1. Relationships among the commonly used equations in radioimmunoassay.
The calibration curves in Figure 2. The ordinates are y or T/B of Fernandez and Loeb, B, B of Hales and Randle, logit transformation of Rodbard, and F/B and B/F of Ekins. T, concentrations or their logarithms are plotted on the abscissa. These linear calibration curves have coefficients of correlation of 0.9997, 0.9996, 0.9996, and 0.9997, respectively. Therefore analytical results of comparable precision are obtainable with experimentally optimized systems.

However, the theoretical interpretations of the corresponding linear equations are very different. As shown previously (5), the intercept of the regression line 1 in Figure 2A represents the sum of the first two terms of the basic equation 18. The slope of this regression line represents the coefficient 1/qV of the third term of equation 18. The intercept of the Hales–Randle plot, line 2 in Figure 2A, should approach 1. The slope should approach the reciprocal of the mass of ligand (sum of radioactive and nonradioactive) contributed by the radioactive ligand reagent. The experimental line of the F/B Ekins plot in Figure 2C is straight and displaced one unit below the T/B line of the Fernandez–Loeb plot but with a numerically identical slope, as shown in Figure 2, A and C. The reason that these lines are parallel and one unit apart is simply that

\[
\frac{F}{B} = \frac{T - B}{B} = \frac{T}{B} - 1 = y - 1
\]

So, for the plot of F/B we can expect a straight line parallel to the y line but displaced one unit below it. However, we cannot reconcile the equation of Ekins with the experimental plot of F/B. The y-intercept of this plot according to the Ekins equation 23 is 1/Kq. The slope is equal to 1/q(1 + (1/Re)), but this term cannot be constant because it includes the variable Re. Therefore it is impossible to superimpose the plot of this equation on the line formed by the experimental points, as is shown in Figure 2C and noted by Ekins et al. (2). The points represent the experimental values of F/B, the straight line is fitted to these points, and the curve corresponds to the Re equation of Ekins. To represent this curve the intercept has been taken equal to the experimental intercept, and the slope equal to 0.208(1 + 1/Re), so that this slope will approach the constant value 0.208 when Re

\[
\text{Fig. 2. } T_4 \text{ calibration curves.}
\]

Pantex immuno T4 Quick Kit
A: y = T/B (Fernandez–Eqn. 18)
B: B/F (Hales and Randle–Eqn. 27)
C: logit B/Be (Rodbard–Eqn. 29)
D: F/B (Ekins–Eqn. 23)

\[
\text{Fig. 3. Vitamin B}_{12} \text{ calibration curves: effects of different amounts of binder protein}
\]

V in µL: 0, 12.5; △, 25; O, 50
A: T/B (Fernandez–Loeb)
B: B/F (Hales–Randle)
C: logit B/Be (Rodbard et al.)

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\[\frac{F}{B} = \frac{T - B}{B} = \frac{T}{B} - 1 = y - 1\]
approaches infinity. Numerically this is equal to:

$$R_{cB} = 0.208 \frac{P}{1 + \frac{1}{R_{cB}}} + 0.411$$

Obviously, the plot of this equation cannot be superimposed on that of

$$F/B = 0.411 + 0.208 P_1$$

The curved Ekins et al. B/F plot in Figure 2D is impractical for theoretical and routine application. Obviously, the physical meanings of the slopes and intercepts of the Hales and Randle and Ekins F/B plots are very different from those of the Fernandez and Loeb plot.

Data on vitamin B₁₂ analysis published in 1975 (5) are plotted in Figure 3 to demonstrate the effects of changing the mass of ligand protein added. As shown in Figure 3A, where results corresponding to three masses of binder are plotted, T/B plots show lower slopes with increasing masses of ligand, as expected from the theory. According to the relationship $1/(V \cdot \text{slope}) = q$, $q$ should remain nearly constant when $V$ is varied. There is about 15% change in $q$ when $V$ is varied by fourfold. For $V$ of 12.5, 25, and 50 µL the slope (ng⁻¹) is 14.2, 7.79, and 4.20 with values for $1/(V \cdot \text{slope})$ of 5.63, 5.13, and 4.76. The lines plotted from the same data in B/B₀ in Figure 3B, or logit in Figure 3C, show no appreciable change of slope or intercept, in conformance with the theory.

Conclusions

Four of the equations used for calculations of radioimmunoassay data give, under adequate experimental conditions, satisfactory linear plots of comparable precision. One of these, the logit-log plot of Rodbard et al., is empirical. The other three are theoretical.

The basic equation of Fernandez and Loeb can be derived solely from the mass-action law. It consists of three terms. By omitting its first term it is possible to derive the equations of Ekins et al. from it, or by omitting its second term it is possible to derive the Hales–Randle equation. The logit-log plot is obtained by making a logarithmic transformation of the Hales–Randle equation.

There are several disadvantages in using either the Hales–Randle equation or the logit-log plot as compared with the basic equation. Changes in the quantity of binder have no effect on slope or intercept, and deviations from linearity cannot be interpreted directly and therefore cannot be corrected readily. This is because the equations do not include the equilibrium constant, the quantity of binder, or the volume of the incubation mixture.

Additional disadvantages of the logit-log plot are the unnecessary logarithmic transformations of the experimental data, the use of a set of standards whose concentrations vary logarithmically to obtain an even distribution of points on the graph, and that the zero concentration standard cannot be represented on the graph.

The B/F plot of the $R_{cB}$ equation of Ekins et al. is too curved to be practical for calculation. The F/B experimental plot is linear, but the theoretical $R_{cB}$ equation is not. Although the F/B plot can be used for practical calculations, the theoretical equation is not acceptable because its slope is not constant, and therefore it is not linear. Because calculation of F/B requires measurement of two variables, there is greater error in using this ratio than in the measurement of T/B.

Although the Fernandez–Loeb equation appears more complex than the other three, it has the simple linear form

$$\frac{T}{B} = y = aP_1 + b$$

It is therefore easily used, either graphically or arithmetically. Linearity is controlled by making variation of the second term negligible as compared with the sum of the first and second terms—e.g., by increasing the volume of the undiluted label reagent U. The slope is controlled by changing the volume of undiluted binder reagent V. The intercept can be controlled by changing the volume U of label reagent. Like the blank in Beer’s law of spectrophotometry (with which it is comparable), the intercept of the basic equation should be as low as possible, so long as curvature is not introduced. If there are changes in the standard curve produced by different lots of reagents or deterioration of reagents, the problems are readily identified and corrected by the use of the T/B plot.

The most nearly complete description of the radioimmunoassay system is given by the basic equation of Fernandez and Loeb, because it incorporates all the fundamental parameters. By omitting certain terms it is reconcilable with the other three equations. In a direct application of the equation of Ekins et al. to calibration curves the quantity of tracer is disregarded, whereas this is the only parameter included in the Hales–Randle equation or in the logit-log plot of Rodbard et al. The basic equation is easily used both for result calculation and trouble-shooting. By giving a formal description and quantitative understanding of an RIA system it provides the potential for optimizing a test or dealing with more complex cases.

References