analyses, 50 T4, 318 TSH, 37 T4-up-take, and 25 T3). The difference between the two strategies was statistically significant (chi-square test, P < 0.005). The same calculation made for the inpatients (533 analyses) gave a reduction to 43.0% as many tests (229 analyses) by the TSH-strategy (87% of the inpatients had TSH in serum between 0.15 and 5.0 milli-int. units/L), and to 52.5% as many tests (280 analyses) by the T4-strategy (chi-square test, P < 0.005).

It is true that the diagnostic specificity of serum TSH as a first-line test is low (1, 4). However, the choice of cutoff limits gives a high diagnostic sensitivity (4) and makes the test useful for screening. The patients described in ref. 1 were from an acute medical facility, the selection for investigation was based on the amount of serum available, and patients treated with thyroidin were included. The results of our study indicate that, in unselected hospital patients and in inpatients thought to have thyroid disease, a strategy with serum TSH as the initial test has certain advantages over a strategy of first applying either serum T4 or FTI. The TSH-strategy requires fewer analyses than the T4-strategy (P < 0.005), and in 84% of the patients (95% confidence limits: 80–88%) one test seems sufficient to identify them as euthyroid. An FTI-strategy would require at least two analyses for each patient (T4 and T3-uptake). Another advantage is the identification of patients at risk of becoming hypothyroid. These patients may have a normal T4 concentration in serum and even a normal value for FTI, but the serum TSH concentration will be increased (5).

We conclude that, for the time being, using the serum TSH assay as the initial test of thyroid function is the best way to decrease the number of thyroid hormone analyses while retaining diagnostic efficiency.

References

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Computing Immunoradiometric Assays

To the Editor:
Haven et al. (1) have recently described spectacularly large measurement differences that can occur if workers attempt to compute immunoradiometric assays (IRMA) by using computer programs specifically designed for radioimmunoassays (RIAs). We offer an explanation for their (1) results and suggest an approach that improves computation of IRMA when either the logit-log linearization method or a four-parameter logistic function is used.

A four-parameter logistic function can be written

\[ B = [a - d/(1 + (X/c)^b)] + d \]  

where B denotes assay response (counts bound); X denotes concentration; and a, b, c, d are the parameters.

Parameters a and d are fitted values of counts bound at X = 0 and X = ∞, respectively, and are commonly known as the "end-point" binding parameters. Equation 1 can be rearranged to the straight-line form

\[ \log(B-d)/(a-B)) = -b \log X + b \log c \]  

which implies that any set of immunoradiometric assay data that exactly fit equation 1 can be converted to lie exactly on a straight line (with slope = -b and intercept = b log c) by simply transforming:

\[ B \rightarrow \log(B-d)/(a-B)) \]

\[ X \rightarrow \log X \]

This linearization technique clearly requires "optimum" estimates of the end-points a and d. Very good approximate values exist for RIAs:

\[ X = 0, a - B_o \] (upper end-point)
\[ X = \infty, d - 0 \] (lower end-point)

where B_o denotes experimentally measured counts bound at zero concentration, and it is assumed counts bound decline to zero at infinite concentration. Substituting these values into equation 2 yields the well-known logit-log equation of Rodbard and Lewald (2),

\[ \log[B/(B_o - B)] = - b \log X + b \log c \]  

which is quite successful at producing near-linearization of RIA calibration data.

The problem with IRMA calibration data is summed up by:

\[ X = 0, a - B_o \] (lower end-point)
\[ X = \infty, d - ? \] (upper end-point)

i.e., unlike RIA, no good estimate is immediately available for counts bound at infinite concentration. Haven et al. (1) investigated three commercial logit-log packages and, in effect, used the following end-point values: a = B_o and d = TOTAL (Micromedic package), where TOTAL denotes total counts added to the assay; a = 0 and d = TOTAL (Hewlett-Packard package); and a = 0 and d = B_max (Iso-Data package), where B_max denotes counts bound for the highest-concentration calibrator. Poor estimates of the lower end-point would be therefore used in two cases, while two markedly different estimates were used for the upper end-point. The severe consequences (failure to achieve adequate linearization) are well illustrated by Haven et al. in their Figure 1.

For workers currently "forced" to use a logit-log package, the appropriate linearizing equation for IRMA is

\[ \log(B - d)/(B_o - B)) = -b \log X + b \log c \]  

e.g., B should be transformed to

\[ \log[(d - B)/(d - B_o)) = \log[(B - d)/(B_o - B)) \]

It is necessary to evaluate equation 4 by using different values of d in the range B_max to TOTAL to get at least a rough approximation to the optimum linearizing value. This is detected as a minimum in the sum of squared residuals about each fitted line, or as a maximum in the correlation coefficients (whichever measure of fit is used by the package). In a stable assay system, experience soon shows roughly where the optimum value of d lies, and it can be refined on a day-to-day basis to the degree considered necessary. Even a rough approximation yields better linearization than simply assuming d = B_max or d = TOTAL. Ideally, workers in this position should seek better software.

End-points a and d are automatically adjusted to their optimum values by fitting a four-parameter logistic func-
tion. However, in our experience (shared by Haven et al.), some computer programs that reliably converge with RIA data may be less successful with IRMA. A poor starting value for the troublesome parameter d is almost always the cause. This is easily overcome by automating the scheme described in the previous paragraph. Our computer program defines 10 equally-spaced trial values of d in the range B_max to TOTAL, then evaluates them in ascending order, using equation 4, until a minimum is found in the sum of squared residuals (best linearization). Usually three to seven evaluations are required. This yields excellent starting values for b, c, and d (B_0 is a good starting value for parameter a). Fitting a few straight lines, in each case to a small number of transformed calibration points, requires trivial computing time, which is more than compensated by rapid convergence in the main curve-fitting process (almost always complete in <4 iterations). This modification, simple to program, requires no knowledge of the main fitting process other than identification of the starting point where initial parameter values are defined.

Convergence problems and even machine "crashes" may still occur on rare occasions when one or more calibration points is badly "out-of-line" (i.e., a technical blunder). In preference to leaving trouble shooting to bench staff, we automate the exclusion of frankly bad correlation data by using a simple extension of the scheme described in the previous paragraph. The evaluation of different values of d is systematically repeated, with each calibration point in turn being excluded from the calculations. The most "deviant" calibration point is identified as the point excluded from the particular fitted line that yields the smallest sum of squared residuals, and a t-test given by Snedecor and Cochran (3) is used to assess whether this point differs significantly from the line. We set a low probability level (P = 0.002) to ensure that a minimum of in-control data are excluded. The main assumptions involved are, first, that equation 4 produces reasonable linearization when d is close to its optimum value, and, second, that B_0 is not itself an outlying value (we routinely use quadruplicate measurement for the zero calibrator). Computing time is equivalent to about four iterations of the main fitting process, not a particularly severe price for objective data editing and improved computational reliability.

Finally, the question of weighting. Linearizing transformations of B (left sides of equations 3 and 4) introduce severe non-uniformity of variance (2). While weighting is unnecessary when the objective is simply to obtain good starting values for b, c, and d, it is essential when significance tests are involved. Following the approach of Rodbard and Lewald (2), appropriate weights for fitting equation 4 are given by

\[ w = \frac{(B - d)(B_0 - d)}{\text{Var}(B)} \]

where Var(B) is the variance of the raw counts bound measurements. Smoothed predicted values of Var(B) are conveniently obtained by estimating the relationship between Var(B) and B (4).

References

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Two authors of the paper being discussed respond:

To the Editor:
We agree with Sadler and Legge that modifying the linearizing equation in the commercial logit-log packages we used for fitting IRMA curves (1) would certainly improve the accuracy of the computation. It is also true that approximating an optimum value for d by the computer method they describe results in fewer iterations being necessary before convergence of the four-parameter logistic function.

However, our premise was that some laboratory personnel do not have the time and (or) the expertise to modify the commercial curve-fitting programs. Often, too, computer program documentation and (or) access does not permit such alteration, and reprogramming may even result in other, undetected errors in computation. We demonstrated that significant errors occurred with some commercial packages while other manufacturers' data-reduction models accurately fit the curves generated by IRMA data. We felt that suggestions that laboratorians choose with care the data-reduction model they will use for IRMA data would be more practical than expecting them to modify equations in existing commercial software.

Reference

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