Enzyme Immunoassay Adapted for Use with a Digital Kinetic Analyzer

To the Editor:

A Micromedic Systems, Inc. MS-2 spectrophotometer equipped with a digital kinetic analyzer and a Micromedic pipet-diluter were found suitable for measuring antiepileptic drug concentrations by the Enzyme Multiplied Immunoassay Technique (EMIT) developed by Syva Research Institute. Printout values of absorbance change per minute supplied by the digital kinetic analyzer, when multiplied by the factor 1333, provide the absorbance change values required for data plots on graph paper supplied with EMIT reagents. The best results were obtained when samples were analyzed in triplicate, but increased cost resulting from replicate assay may be partly offset by use of half volumes for samples and reagents, with little loss of accuracy. Adaptations of the EMIT technique to centrifugal analyzers have been described previously.

For assay with the MS-2, 1-ml plastic cups are used and the spectrophotometer settings are as follows:

- Wavelength: 340 nm
- Light source: visible
- Mode: absorbance
- Temp: 30 °C
- Automatic switch: on
- Sampling interval: 15 s
- Mode: +E

Either the volumes recommended in the EMIT procedure or half of the recommended volumes may be used. Measurements are performed in triplicate. Set the timers to draw up 0.7 to 0.8 ml for full-sized samples or 0.35 to 0.4 ml for half-volumes. Maintain a uniform timing sequence when handling each sample.

For each standard and sample, calculate the average of the second, third, and fourth (ΔA/min)ₚ printout readings. Then calculate the average of the three replicate averages. Subtract the average (ΔA/min)₀ for the zero concentration standard from the replicate average. These differences, (ΔA/min)ₚ - (ΔA/min)₀, multiplied by 1333 are plotted as ΔA = ΔA₀ on the AED graph paper supplied. The factor 1333 arises from the fact that, for the EMIT system, milliabsorbance differences are measured for 80 s, whereas for the MS-2 the absorbance differences calculated by the instrument are for 60 s. (The factor 1.333 may be set on a dedicated channel of the MS-2 so that readings are multiplied by this factor.)

Calculations reveal that the standard deviation for the zero concentration standard is typically 0.0030 ΔA/min, but is only 0.0008 ΔA/min for the other standards and samples. Thus the large uncertainty in the zero-concentration value determines most of the uncertainty in sample concentrations. This may be seen when values are put into the relationship

\[ (A ± a) - (B ± b) = (A - B) + \sqrt{a^2 + b^2} \]

where A and B are the printout readings from the spectrophotometer for samples with and without drugs, respectively, and a and b are the respective errors in A and B.

The results obtained by calculations that employed averages of the averaged second, third, and fourth printout values for three replicates were better than values based upon smaller numbers of data points. For 16 samples with concentrations distributed over the EMIT assay range, the average CV was 3.4%, and the largest CV for this group was 7.1%. Values obtained on two control materials analyzed by either the half-volumes and full-volumes procedure are in good agreement, but were as much as 7% lower with smaller volumes. The values obtained by this procedure have an average percent deviation of 6% from values for standard sera having weighed-in drugs. For 35 Pippenger (4) survey samples with values lying in the recommended range for EMIT assay, the EMIT values found have an average percent deviation of 7.4%. These values compare favorably with the relative standard deviation values of 15% given in the product literature.

References


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Radioimmunoassay Equations Derived

Ed. note:

The authors of a correction [Clin. Chem. 23, 2343 (1977)] to the first equation on page 152 of a review on radioimmunoassay [19, 146 (1974)] have received so many requests for the detailed derivation that we have decided to publish it in full. What follows is excerpted from a letter received August 15, 1977, from these authors (Drs. C. L. Pappkin (Imaging Division) and John E. Hammond (RIA-Endocrine Laboratory) of the School of Medicine, The University of North Carolina, Chapel Hill, N.C.

The equation is incorrectly given as:

\[ K[A_g] - [Ag] \times \frac{[Ag]}{[Ag] + [B/F] + 1} \]

whereas the correct equation should read:

\[ K[A_g] - [Ag] \times \frac{[Ag]}{[Ag] + [B/F] + 1} \]

The detailed derivation, starting from the generally accepted antigen-antibody equilibrium equation and assuming validity of the law of mass action, is as follows:

\[ \text{Ag + Ab} \rightleftharpoons k \text{Ag Ab} \]

where

\[ k = \text{rate constant for association} \]
\[ k_1 = \text{rate constant for dissociation} \]
\[ K = \frac{k}{k_1} = \text{equilibrium or affinity constant} \]