Automated Solid-Phase Assay for Estimation of Thyroxine Binding Capacity in a Screening Program for Congenital Hypothyroidism

To the Editor:

We recently described a method for evaluating the binding capacity of thyroxine-binding globulin (TBG) by use of samples spotted on filter paper in a mass-screening program for congenital hypothyroidism (1). To automate our screening program fully, we have now adapted our method to the “Systems Concept 4” (Micromedic Systems, Horaham, PA 19044), using their Neonatal T4 Kit, a system that we have been using for thyroxine (T4) measurement for the last two years. Particularly showing a low thyroxine on filter paper but a normal thyrotropin concentration, two 6-mm spots on filter paper are placed in 800 µL of barbitral buffer (pH 8.6, 0.75 mol/L) in tubes coated with anti-T4 serum and allowed to elute overnight. The omission of normal rabbit serum and 8-anilino-1-naphthalene sulfonate from the buffer allows competition for the T4 molecule between proteins eluted from the spots and the anti-T4 antibody. The next day, radiolabeled T4 (~25 000 cpm in 200 µL of barbitral buffer) is added to the tube and the mixture is incubated for 30 min at 37 °C, as for the regular assays. The contents of the tubes are emptied out, the tubes are gently rinsed with distilled water, and the radioactivity in the empty tube is counted. Results are expressed as percent binding of the 125I-T4 to the coated tube, the “100%” tubes (quadruplicate) being tubes without eluate. In contrast to our previously reported method involving charcoal absorption (1), samples containing low concentrations of TBG will yield high values. The within-assay CVs for normal and high values were respectively 6.0 and 6.7%. On the other hand, the between-assay CVs were 6.9% for normal values and 6.7% for high values. The mean binding for our normal population (n = 186) was established at 18.3% (SD 3.5%), compared with the low TBG (n = 20) binding of 40.3% (SD 3.4%). The correlation (r) between values obtained by this method and our previously reported method was 0.73 for 64 samples, including eight samples from patients with low TBG. Furthermore, with a cut-off point for recall established at 28%, the false-positive rate of recall is only 1%.

Reference

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Iodination of Antiferritin by the Method of Bolton and Hunter

To the Editor:

In 1977, I reported having used the Bolton and Hunter technique (1) for labeling purified antibody with 125I-labeled hydroxyphenyl propionic acid N-hydroxysuccinimide ester, for use in the immunoradiometric assay for serum ferritin (2). The conditions for the iodination procedure, the uptake of 125I, and the stability of the labeled preparation which I observed with the method were cited in this article.

Recently, Alvarez-Hernández and Loria (3) reported the use of the Bolton-Hunter reagent for labeling antiferritin, together with the claim that theirs is the first report of the method for this purpose. They failed to include any of the conditions of the iodination, although their observed uptake of 125I and stability of the iodinated antibody were similar to that which I reported.

The significance of my earlier report, which these authors overlooked, was not in the labeling of antiferritin specifically for use in a single immunosassay, but in the application of a new technique for iodinating large proteins. The advantages offered by the method are simplicity and mild conditions which eliminate oxidation and reduction reactions in the presence of the protein to be labeled. Since my report, the method has been applied to the iodination of ferritin, a protein larger than IgG antibody, with subsequent improved storage characteristics as compared with ferritin labeled with oxidation methods (4, 5).

References

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The authors of the communication in question respond:

To the Editor:

The omission of the paper by Gonyea is the outcome of a perhaps growing problem in computer technology, as a five-year retro-search under several words (either label, antibody, antinodies, immunoglobulins, and IgG coupled to either antiferritin or Bolton and Hunter reagent) failed to detect her publication. The computer was linked to the BIOSIS system in the U.S. Our apologies to Dr. Gonyea for this inadvertent and unfortunate omission.

In regard to her questions on the iodination procedure: 50 µL of a 50% suspension of Sepharose 4B coupled to human ferritin is incubated overnight with 500 µL of rabbit serum containing antiferritin, to yield an immunosorbent with 50–90 µg of adsorbed antiferritin. The immunosorbent is incubated overnight with 500 µCi of air-dried Bolton/Hunter reagent. It is washed three times with borate buffer, and incubated for 30 min with 2 mL of 3 mol/L KSCN containing 50 g of bovine serum albumin per liter. The supernate is dialyzed against borate buffer. The final preparation is aliquoted and stored at −20 °C.

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