1. Determination of the "in-house interassay precision profile curve" (9). Five serum pools with TSH concentrations corresponding to the subnormal (at least two pools) and normal range are included in eight to 10 consecutive routine assays to assess the precision actually achieved by a laboratory over the normal and subnormal range.

2. Graphical assessment of the "in-house lower limit of interassay quantitative measurement" or "lower limit of the assay working range" under routine conditions: this is determined from the intercept of the interassay CV (y-axis) equal to 10% or 15% with the "in-house interassay precision profile" curve.

3. Determination of the "in-house reference range" for euthyroid individuals.

4. Assay classification: an assay can justifiably be called "sensitive" and deserves further clinical trials in hyperthyroid patients if and only if the "lower limit of quantitative measurement" falls clearly below the normal range. Or, in other words, a clear-cut, complete separation of hyperthyroid and euthyroid TSH values can be achieved only if the inherent sensitivity of the TSH assay and the technical expertise of the laboratory staff are such that TSH concentrations below the normal reference interval are still reproducible with a CV of <15%.

For instance, the enzyme immunoassay (EIA) from Abbott Laboratories as performed in our laboratory under routine conditions met the fundamental requirement for a "sensitive" assay (Figure 1), and subsequent clinical investigations have confirmed that initial conclusion. By contrast, various other TSH assays, also designated by their manufacturers as "sensitive," have failed in our hands to meet the criteria, suggesting that they cannot be used to diagnose hyperthyroidism.

References

Monika F. Bayer
Diiv. of Nuclear Med.
Dept. of Diagnostic Radiology & Nuclear Med.
Stanford Univ. Sch. of Med.
Stanford, CA 94305

Low-Ultraviolet "White" Fluorescent Lamps Fail to Protect Pyridoxal Phosphate from Photolysis

To the Editor:

The photodegradation of vitamin B₆ has been widely recognized and studied in considerable detail (1, 2). Since Ang (3) demonstrated that yellow incandescent or yellow fluorescent lights prevented significant deterioration of vitamin B₆, we have routinely used such lighting in our laboratory. However, it is somewhat uncomfortable to work continuously in yellow light, and we have encountered some inconveniences as a result of the false colors produced by the yellow lighting. "White" fluorescent lamps, with minimal output in the 290–380 nm range, have been produced (Color-Gard 50; Luxor Lighting Products, Inc., Lyndhurst, NJ). Because of the general assumption that ultraviolet wavelengths are most responsible for the deterioration of vitamin B₆ compounds, we tested the effects of these lamps on solutions of pyridoxal phosphate.

Solutions of pyridoxal phosphate, 10 mg/L in various solvents (see Table 1), were exposed to the following light at a distance of 175 cm for 21 h: four unshielded "cool-white" fluorescent tubes, the same tubes with translucent plastic shielding, four 40-W low-ultraviolet fluorescent tubes, and four "gold fluorescent" tubes. The illumination striking the pyridoxal phosphate solutions from each source (measured with a General Electric type 214 light meter) was 366, 430, 452, and 355 lx, respectively.

For each trial we placed 1 mL of each pyridoxal phosphate solution in a low-actinic glass test tube as a control and 1 mL in clear test tubes, in triplicate. The low-actinic glass minimized photodecomposition under all lighting conditions and served as a control for chemical decomposition. All tubes were stoppered, placed in wire racks, and exposed to light in a windowless room for 21 h at room temperature. The absorbance was read before and after exposure at the wavelength of the maximum ultraviolet absorption (Table 1). The pyridoxal phosphate concentration in each sample was also determined by liquid chromatography (4).

Only the gold fluorescent lighting prevented pyridoxal phosphate deterioration in water or in neutral or basic solutions in clear test tubes: virtually 100% of the pyridoxal phosphate was accounted for, as determined by comparison with results for the solutions in the low-actinic glass control tubes (Table 1). Under each of the other three lighting conditions there was substantial destruction of the pyridoxal phosphate. Acidic conditions tended to protect against photolysis in all lighting, possibly because the wavelength of maximum absorption is shifted down to 294 nm at pH 4. Under neutral and alkaline conditions the maximum absorption is at about 390 nm. While the output of the low-ultraviolet lamps is negligible below 380 nm, the manufacturer's literature indicates an output of 70 µW/10 nm lumen over 380 to 400 nm; the yellow fluorescent tube was 'white' but emitted very little light below 380 nm.
440 nm. The fact that there is output between 380 and 400 nm may explain why these lamps did not protect against photolysis under neutral and alkaline conditions. We conclude that the low-ultraviolet lamps can not be used in place of yellow lamps to protect against photolysis of pyridoxal phosphate.

This work was supported in part by grant no. 85-CRCA-1-1554 from the U.S. Dept. of Agriculture Competitive Grant Program.

References

Wayne E. Schaltenbrand
Maureen S. Kennedy
Stephen P. Coburn

Biochem. Dept.
Fort Wayne State Developmental Center
Fort Wayne, IN 46835

Discordant Inter-Kit Results in Immunoradiometry of Serum Thyrotropin

To the Editor:

In accord with the negative-feedback regulation of secretion of thyrotropin (TSH) by the thyroid hormones, TSH production is suppressed (or stimulated) in states of increased (or decreased) thyroid function. A sensitive TSH assay is therefore useful in differentiating normal, thyrotoxic, and hypothyroid patients and in assessing the efficacy of treatments of patients with abnormal thyroid conditions (1).

Some manufacturers have used an immunoradiometric assay (IRMA) technique that is more sensitive in kits with alkaline buffer than in TSH assay, several of which have been evaluated: Boet-Cellteg Suncro- sep IRMA-TSH (2, 3), TSH3MAIA clone (4-6), Tandem-R TSH (7), Delfia hTSH (8), Abbott TSH-RIA (9), and Enzymun-Test TSH (10). I used the TSH3MAIA clone kit product (Sero- nantar Diagnostics, Braintree, MA) to assay more than 2500 patients’ samples between March 1984 and April 1986 and this assay has been in routine use since April 16, 1986; TSH concentrations in more than 4500 patients’ samples have been determined with this kit. More than 1100 of these samples were stored and the TSH has been found to be stable at 20 °C for at least 15 months. The value of the coefficient of correlation (r) between fresh and frozen samples is 0.97 (n = 38). The minimum TSH detectable is 0.1 milli-int. unit/L. Between-assay CVs, calculated from results obtained with this kit by nine technologists, were 5.0% (n = 55), 7.8% (n = 121), and 8.8% (n = 91), respectively, for three control samples with mean values of 4.8, 2.1, and 1.4 milli-int. units/L. Technologists have been satisfied with the performance of this product.

Unfortunately, erroneous TSH results for a few patients’ samples, noticed in this and another laboratory (6), prevented me from using this assay as a first-line test for thyroid function and led me to evaluate a different product, a solid-phase TSH IRMA kit (Pacific Biotech, Inc., San Diego, CA). The assay sensitivity of this product is stated by the manufacturer to be 0.03 milli-int. unit/L. Between-assay CVs obtained by this laboratory were 10.5% and 5.8% for two control samples with mean values of 0.8 and 6.5 milli-int. units/L, respectively, in 27 assays. TSH concentrations in 145 thyrotoxic patients’ sera selected from the stored samples were determined with both kits; all contained TSH concentrations <0.5 milli-int. unit/L. Identical results were obtained for 130 patients’ samples, 125 being <0.1 milli-int. unit/L and five between 0.1 and 0.3 milli-int. unit/L. For the other 15 samples, the difference between the two results was <0.2 milli-int. unit/L. For sera from 118 normal and 45 primary hypothyroid patients, linear regression comparison of results by TSH3MAIA clone kit (y) and the Pacific Biotech TSH IRMA kit (x) gave the following correlation: normal: y = 1.07x + 0.28 (r = 0.97); primary hypothyroid: y = 0.90x + 5.62 (r = 0.94).

We have been satisfied with the comparability of the two assay procedures in most cases. However, we observed discrepant results for sera from several patients, two of whom are shown in Table 1. Patient A.M., a 75-year-old woman in the Home Care Unit of this hospital, who had above-normal results for T4 and T3 uptake tests in sera on three occasions, had a TSH concentration in serum that far exceeded 50 milli-int. units/L as determined with the TSH3MAIA clone kit. To validate this TSH value, we diluted the patient’s sample and re-assayed, but found no parallelism between the TSH values for the original and diluted serum, indicating the presence of an interfering factor(s). This sample was stored at -20 °C and re-assayed 11 months later with both kits. The TSH3MAIA clone kit gave an equivalent value of 22.3 milli-int. units/L, significantly lower than the value determined previously but still far greater than the TSH concentration for a normal individual. The Pacific Biotech kit, however, gave a TSH value of 0.1 milli-int. unit/L.

Patient G.A., a thyrotoxic 69-year-old woman, showed between normal and borderline increased concentrations of TSH by TSH3MAIA clone procedure, and no parallelism of results was demonstrated in diluted and original serum drawn on September 15.