were analyzed during the same period. All showed increased TSH (43–235 mIU/L). Four showed clearly decreased T4 [1.2–23.8 nmol/L (0.1–1.85 μg/dL)]. Another two had T4 concentrations below our cutoff of 30.8 nmol/L (2.4 μg/dL), and only one had a T4 concentration within the reference interval [67 nmol/L (5.2 μg/dL)]. This is in agreement with literature data that ~10–20% of all CH cases with increased TSH concentrations are not accompanied by low T4 (1). Interestingly, the ratio of TSH to T4 was an excellent indicator for CH in neonates because deviations in TSH as well as T4 values will yield highly increased ratios of TSH to T4. For healthy newborns, we observed ratios of 0–4, whereas our seven patients as well as abnormal controls showed values >20.

In summary, our modified multi-analyte assay for TSH and T4 simplifies the analytical protocol of the procedure to allow easier handling and more rapid analysis. Furthermore, future automation of the process becomes an option, which is indispensable for the high-throughput screening usually required in all larger screening laboratories. LabMap technology provides an alternative to mass spectrometry for the simultaneous analysis of metabolites present in samples at very low concentrations. Other endocrinologic metabolites, e.g., 17-hydroxyprogesterone, a marker for congenital adrenal hyperplasia, can also be incorporated in the analytical protocol. This would allow the simultaneous analysis of all endocrinologically relevant metabolites for newborn screening in one analytical run.

References


Zoltan Lukacs1* Caroline Mordac2 Alfried Kohlschütter1 Rudolf Kruithof2
1 Department of Pediatrics Metabolic Laboratory University of Hamburg Martinistrasse 52 20246 Hamburg, Germany
2 RA Laboratories Ltd. Mill Lane Tayport DD6 9EL, Scotland

*Author for correspondence. Fax 49-40-42803-6717; e-mail lukacs@uke.uni-hamburg.de.

Commentary on: Use of Microsphere Immunoassay for Simplified Multianalyte Screening of Thyrotropin and Thyroxine in Dried Blood Spots from Newborns

To the Editor:

In their letter, Lukacs et al. present an interesting modification and improvement of the procedure described previously by Bellisario et al. (1), using the Luminex Xmap system to simultaneously assay for thyroxine (T4) and thyrotropin (TSH) in a Guthrie specimen. Their innovation builds on the earlier work and, importantly, negates the necessity for multiple wash steps while maintaining acceptable sensitivity and specificity. This will greatly facilitate automation of these tests, an important aspect for newborn screening programs, in which thousands of specimens are processed each day. The concept of simultaneous measurement of T4 and TSH enhances the specificity of hypothyroidism screening in newborns, at the same time reducing the workload by combining two tests into a single procedure. Their comments concerning the use of a TSH:T4 ratio are interesting and worthy of follow-up.

The use of ratios such as this has been helpful in increasing the specificity of other newborn assays, such as for phenylketonuria (PKU) (2). With the addition of a bead-based test for 17-hydroxyprogesterone, the serum marker for congenital adrenal hyperplasia, and immunoreactive trypsinogen, the serum marker for cystic fibrosis, to the T4/TSH assay, a complete endocrine panel for newborn screening could be accomplished in this system in a single assay tube. This multiplex testing offers newborn screening programs tremendous opportunities for expanding the screening panel without increasing the workload.

References


Kenneth A. Pass
Department of Health
Wadsworth Center
Albany, NY 12201-0509

Comparison of Two Third-Generation Parathyroid Hormone Assays

To the Editor:

Parathyroid hormone (PTH) is an 84-amino acid peptide secreted by the parathyroid glands that is important in regulating the extracellular calcium concentration in the body. Because this hormone is rapidly metabolized into fragments, its measurement has always been problematic (1).

During the last few years, it has been shown that the current intact PTH, or second-generation, assays will cross-react with a large C-terminal fragment, PTH (7–84), which lacks the six N-terminal amino acids (2, 3). Because of the lack of specificity in these second-generation systems, falsely increased PTH values have been observed (3). New third-
generation PTH tests do not detect PTH (7–84) and therefore provide a more accurate measurement of bioactive PTH.

The specificity of third-generation parathyroid hormone assays is important, particularly when evaluating patients with end stage renal disease. In these patients, the clearance of C-terminal PTH fragments is dramatically impaired, leading to increased concentrations of PTH (7–84) fragment. PTH (7–84) has been found to have a weak anticalcemic effect (4, 5). The control of calcium concentrations in dialysis patients poses considerable difficulties as illustrated by the finding that 90% of the patients in a 1995 study had osteodystrophy, either adynamic or low (52%) bone turnover or increased (38%) bone turnover disease (6).

Two third-generation PTH assays that claim PTH (1–84) specificity are the CAP (cycloase-activating PTH) assay from Scantibodies Laboratory and the Bio-Intact PTH assay from Nichols Institute Diagnostics. The Scantibodies CAP assay measures whole (1–84), intact PTH by a manual 125I two-site IRMA. The specificity of the third-generation CAP assay is conferred by the use of an antibody directed toward the (1–4) amino acid epitope of the PTH molecule. The Nichols Bio-Intact assay is an automated PTH test performed on the Nichols Advantage Speciality System instrument. The methodology consists of a two-site chemiluminescence immunoassay, with one antibody directed toward the first six N-terminal amino acids. Because one antibody of each pair is directed toward the (1–6) N-terminal epitope of PTH, both assay systems should show the desired specificity.

To compare the two assays, we assayed 20 EDTA plasma samples, 10 from healthy volunteers and 10 from renal hemodialysis patients, with the Nichols Advantage Bio-Intact PTH, the Scantibodies CAP, and the Scantibodies total PTH (second-generation) assays.

Deming regression analysis comparing Nichols Advantage and Scantibodies CAP assays (Fig. 1) yielded a slope of 0.904, which was statistically different from 1.00 (P = 0.001); the y-intercept (13.8 ng/L) also differed statistically from 0 (P = 0.001). Although the differences from identity were statistically significant, the Scantibodies CAP and Nichols Bio-Intact assays were still closely correlated. The slope of the regression line between the two third-generation PTH assays was 0.904, indicating that the Scantibodies CAP assay and the Bio-Intact PTH assay using the Nichols Advantage instrument.

Data include specimens from both healthy and uremic individuals. Equation for the regression line (solid line): y = 0.904x + 13.8 ng/L; R² = 0.987; n = 20. The dashed line is y = x.

At low PTH concentrations, the Nichols Advantage gave higher PTH results than those obtained with the Scantibodies CAP. The reason for this may be standardization and/or standard matrix differences, but it does not appear to be related to impaired specificity because at higher concentrations, the Advantage reported lower PTH concentrations than those obtained with the Scantibodies CAP.

The reported interassay CV of the CAP assay is 8.3% at 31 ng/L and 3.4% at 359 ng/L over nine different batches (7). The interassay CVs for the Nichols Bio-Intact assay run on two different instruments on 5 different days were 2.7% at 20.8 ng/L, 2.9% at 143.2 ng/L, and 3.7% at 470.9 ng/L.

Unpublished data from our laboratory have shown that the Bio-Intact PTH assay does not cross-react with the PTH (7–84) fragment. We conclude that although there are minor differences between the assay results, they should produce clinical agreement when used for diagnostic purposes.

References


*Author for correspondence.