Radioimmunoassay of Serum Parathyrin: Comparison of Five Commercial Kits

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We compared serum parathyrin radioimmunoassay data obtained with five commercially available kits for normal subjects, patients on dialysis, and patients with hyperparathyroidism. The Cambridge Nuclear kit gave low results, did not distinguish the dialysis-group sera from the normal sera, and had the greatest CV (56%) both inter- and intra-assay. The Immuno Nuclear PTH-II kit gave more satisfactory clinical data than their PTH-C-terminal kit; however, as with most procedures involving a substantial and variable nonspecific binding correction for each serum, parallelism between samples and the standard curve was poor, turnaround time slow, and inter-assay CV high (39%). Unlike the other kits, the Nichols and the Iso-Tex products depend on a direct methodology; they displayed good parallelism and distinguished among our three populations. A Scatchard plot of results with the Nichols kit was closest to the ideal linear model; the Iso-Tex kit had the shortest turnaround time.

**Additional Keyphrases:** dialysis • hyperparathyroidism • "kit" methods • C-terminal parathyrin • hormones

Traditionally, measurement of parathyrin (PTH) in blood has been considered difficult, so that most clinical assays for this hormone were done in a few reference laboratories (1, 2). The raising of antisera specific for and sensitive to human PTH continues to be more capricious than for most of the human peptide hormones. Human PTH is rapidly degraded after it leaves the gland; the biologically active 84-amino-acid molecule has a short half-life (t₁/₂ = <10 min), whereas a C-terminal (and biologically inactive) portion of the molecule that remains in the circulation has a half-life measured in hours (3, 4). In clinical practice, RIA procedures involving antisera specific for the C-terminal portion of the PTH molecule have been the most valuable for recognition of primary hyperparathyroidism. Several kits for this assay have become available. Here we compare five of them.

**Materials and Methods**

**Kits**

The five kits tested were from Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA 01865; two from Immuno Nuclear Corp., Stillwater, MN 55082 (the PTH-II and C-Terminal kits); Iso-Tex Diagnostics, Friendswood, TX 77549; and Nichols Institute Diagnostics, San Pedro, CA 90731. Each of these kits involves use of a bovine PTH standard, ¹²⁵I-labeled PTH as tracer, and a second-antibody separation technique.

**Cambridge Nuclear** (kit size: 100 tubes; current cost: $225/100 tubes). All reagents are supplied lyophilized and require reconstitution. Serial dilutions are used to generate a six-point standard curve ranging from 1.25 to 50 μg/L. Because this kit requires "zero point" determinations on each specimen—i.e., a measure of label displacement when PTH is 0.0 μg/L in that specimen—a "stripping" or absorption step, in which PTH is removed from one aliquot of the serum sample before assay, is necessary. Consequently, the amount required for a single assay is 800 μL, although the manufacturer claims that as little as 350 μL can be used when the sample size is limited. A special rotary mixer is required for the stripping step. The ovine antibody recognizes the C-terminal portion of the 84-amino-acid hormone. A 39-h total incubation, in four separate periods, is required. Isotonic saline, needed for the assay, is not provided in the kit. After reconstitution, kit components are stable for one week at 2–8 °C, or for one month at −20 °C. The exception is the PTH stripping complex, which cannot be frozen but can be stored safely at refrigeration temperatures for four to six months.

**Immuno Nuclear PTH-II** (kit size: 125 tubes; current cost: $250/125 tubes). All reagents in this kit require reconstitution. Preparation of a six-point standard curve ranging from 0.39 to 12.5 μg/L depends on serial dilution from a 12.5 μg/L stock standard. This kit, as does the Cambridge Nuclear kit, depends on a PTH "stripping" step. Because of this, the sample requirement here too, for a single assay, is preferably 600 μL, with a minimum of 350 μL. This system utilizes a chicken antibody directed towards the 44–68 amino acid sequence of the PTH molecule. The kit also calls for four separate incubation periods; however, the investigator is given the option of varying incubation duration to accommodate work schedules. Initially, the sample may be incubated with a Sepharose stripping complex at 2–8 °C for a minimum of 16 h or at ambient temperature for at least 2 h. Incubation time for samples (absorbed and original sera) and standards plus anti-PTH serum is also flexible, ranging from a minimum of 24 h, to a maximum of 72 h at 2–8 °C. The third incubation, with tracer, must be exactly 24 h at 2–8 °C, followed by a final incubation with second antibody and polyethylene glycol for 2 h at ambient temperature or 16–72 h at 2–8 °C. For this study we incubated samples with the stripping complex overnight at 4 °C on a rotary mixer; with the first antibody 24 h at 4 °C; with the tracer exactly 24 h at 4 °C; and with the second antibody for 16–24 h at 4 °C. After reconstitution, all components when stored at −15 °C or lower are stable for an extended period of time; however, the ¹²⁵I-labeled PTH expires in approximately four weeks. Storage and stability recommendations do not hold true if tubes, sera, and reagents are not kept at 2–8 °C at all times. The entire assay must be performed on crushed ice; however, centrifugation may be at room temperature. This kit also requires a special rotary mixer for the stripping step.

**Immuno Nuclear C-Terminal:** (kit size: 125 tubes; current cost: $375/125 tubes). Reconstitution of all reagents is nec-

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necessary; however, the six standards, covering a range of 0.312 to 10.0 \( \mu \text{g/L} \), come preweighed so that serial dilutions are not required. The assay is based on a chicken antibody, which reacts with the 65–84 sequence of human PTH. Sample requirement for a single assay is 50 \( \mu \text{L} \). Minimum incubation time is 22 h. After reconstitution, if stored at \(-15^\circ\text{C}\) or lower and handled to avoid freeze–thaw cycles, as specified, the kit components are stable for an extended period, except for the \(^{125}\text{I}\)-labeled PTH, which expires in about six weeks.

**Iso-Tex:** (kit size: 50 or 100 tubes; current cost: $225/100 tubes). This kit requires reconstitution of all reagents; however, serial dilutions are not necessary for the six-point standard curve, which covers a range of 0.375 to 12.0 \( \mu \text{g/L} \). All critical reagents are color-coded. The guinea pig antitbody recognizes the C-terminal portion but not the 1–34 amino-terminal portion of the human PTH molecule. The sample needed for a single determination is 200 \( \mu \text{L} \), minimum incubation time 15 h. Although kits are shipped at ambient temperatures, Iso-Tex recommends storage at 2–4 \( ^\circ\text{C} \). Upon reconstitution of vials, reagents are aliquoted and stored frozen at \(-20^\circ\text{C}\).

**Nichols:** (kit size: 100 tubes; current cost: $240/100 tubes). Most of the reagents require reconstitution; the immunoprecipitant and buffer are ready to use. The tracer and antisera are color-coded. Six individual standards are supplied, to produce a standard curve ranging from 0.1 to 20 \( \mu \text{g/L} \). The antigenic recognition sites of the guinea pig antitbody antiserum are in the 53–84 portion of the PTH peptide.
anti-bPTH is incubated with standards or samples and labeled antigen for 48 h at 4 °C, then for 3 h more at the same temperature while the bound antigen is precipitated with second antibody. Sample requirement for a single determination is 100 μL. Nichols recommends that the entire assay be set up at 4 °C with all reagents and patients' sera being kept on ice during that time. All reagents should be stored frozen. Reconstituted components are stable at 2–8 °C for one month, or at -20 °C for six to eight weeks. The 125I-labeled PTH is stable at 2–8 °C for 14 days.

**Procedures**

We have at various times used or tried to use kits from each of these five manufacturers for the routine determination of PTH in our laboratory. The study results concur with our experiences on these occasions; however, the data and comparisons presented here are based solely on specimens collected for the purpose of this study. Each specimen was assayed with each of the five systems, and each manufacturer's system was represented by two or more kits.

Blood was obtained from 42 consenting subjects: 21 normal controls, 10 patients on dialysis, and 11 patients in whom primary hyperparathyroidism was subsequently confirmed by surgery. Blood was kept on ice until it reached the laboratory. It was then centrifuged in a refrigerated centrifuge and the serum aliquoted into several glass tubes. The serum was stored frozen (-20 °C) until the assays were run. In no case did any specimen undergo more than one freeze-thaw cycle before assay.

All PTH assays were performed in accordance with the kit manufacturers' instructions.

Total calcium of all samples was determined with an AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, NY 10591).

Inter-assay reproducibility for each kit was assessed with an in-house control obtained by pooling serum from 25 to 30 subjects. This control, like the samples, was aliquoted and frozen, avoiding repeated freeze–thaw cycles. It was assayed in triplicate four times with the Cambridge Nuclear and Immuno Nuclear kits, 11 and 12 times, respectively, with the Nichols and Iso-Tex kits. Each sample and control, including high and low kit controls, within a given run was assayed in triplicate. Intra-assay reproducibility was derived from these replicates. Parallelism of serum samples and standard curves was tested with the in-house control. Sensitivity of the assays and their ability to distinguish among the three groups of subjects were considered, as was cost per assay. Recovery studies were undertaken only with the Nichols and Iso-Tex.
kits. Samples for recovery studies were prepared by addition to the in-house control of approximately 1 µg of standard per liter from these two kits. Each standard, control, and PTH-fortified control was then assayed with the two kits. Where practical—that is, where equilibrium conditions are met in the assay methodology—Scatchard plots were obtained.

**Results and Discussion**

Technical information including standard curves, parallelism between standards and sera, nonspecific binding, maximum binding, and inter- and intra-assay reproducibility (CV) for each of the kits is summarized in Figure 1.

Reported as mean ± 1 SD, results for the in-house control assay (µg/L) were as follows in the five systems: Cambridge Nuclear 0.98 ± 0.90 (n = 12); Immuno Nuclear PTH-II 1.39 ± 0.22 (n = 12); Immuno Nuclear C-Terminal PTH 1.38 ± 0.46 (n = 12); Iso-Tex 2.15 ± 0.45 (n = 35); and Nichols 1.64 ± 0.34 (n = 33). The mean ± 1 SD for the sera from the normal subjects was 0.20 ± 0.21, 0.37 ± 0.16, 0.67 ± 0.79, 0.96 ± 0.28, and 1.15 ± 0.18 µg/L for the Cambridge, Immuno Nuclear PTH-II, Immuno Nuclear C-Terminal PTH, Iso-Tex, and Nichols kits, respectively. The numerical differences among the various kits, which, as will be seen, become even more pronounced among the subjects with renal failure, is a consequence not of poor analysis but of the difference in the antigenic determinants recognized by the different antibodies. The “C-terminal fragment” we speak about is really a spectrum of C-terminal fragments, including the intact 84-amino-acid hormone as well as a series of degradation products. The numerical answer obtained with a given kit depends on the specific amino acid residues with which the particular antisera react. It is possible to get clinically valid assays reporting out different absolute values. By the same token the normal and abnormal ranges and the numerical data obtained with one kit will not necessarily be identical with those obtained with another kit. Clinical correlation studies are presented in Figure 2, where serum PTH concentration is plotted vs total calcium. Scatchard plots for the Iso-Tex and Nichols kits, which in our hands yielded the best clinical data, are shown in Figure 3.

The Cambridge Nuclear kit (Figures 1 and 2) contains an antibody that appears to lack both sensitivity and specificity. The useable portion of the curve spans about 1.1 to 50 µg of PTH per liter of serum, whereas from the analyst’s point of view a system measuring from 0.25 to 25 µg/L is desirable. Based on the data shown in Figure 1, the undiluted serum, unstripped, has the PTH equivalent of 6.2 µg/L; stripped, 5.5 µg/L. From this, the technologist is to conclude that the PTH concentration in the specimen is 0.7 µg/L. Obviously, PTH constitutes only 11.3% of everything with which the antisera reacts in the original specimen. The intra- and inter-assay reproducibility and the clinical correlations reflect this problem. As one may anticipate, results with this kit improve as the percentage of PTH in the mixture recognized by the antibody increases. Primary hyperparathyroid cases with high serum PTH concentrations, therefore, are distinguished fairly adequately. Borderline distinctions between normal and primary hyperparathyroid patients, however, are blurred, and the anticipated negative correlation between normal serum PTH and serum calcium concentrations is extremely weak (−0.009). That there is virtually no discrimination between dialysis patients and normal control subjects is intriguing in view of the claim that the antibody recognizes the C-terminal portion of the PTH molecule. This fragment is generally grossly increased in dialysis patients, probably as a result of two processes: secondary hyperparathyroidism and poor clearance of the fragments. Because of the interaction of these two factors, none of the C-terminal kits accurately evaluate the parathyroid gland status of the dialysis patients.

The Immuno Nuclear PTH-II kit (Figures 1 and 2) also suffers from lack of specificity, although the sensitivity is better than that of the Cambridge Nuclear kit. The correlation between normal serum PTH concentrations and the respective serum calcium concentrations is −0.144. Sera from patients on dialysis are clearly categorized. Nonetheless, the

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**Table 1. Cost of Analysis for 10 Patients in One Run**

<table>
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<tr>
<th></th>
<th>Cambridge</th>
<th>Immuno Nuclear</th>
<th>Iso-Tex</th>
<th>Nichols</th>
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*As of May 1981.
diagnosis of primary hyperparathyroidism with this kit depends to a disproportionate degree on the concentration of serum calcium.

The Immuno Nuclear C-Terminal kit (Figures 1 and 2) gives a good standard curve over a useful range of PTH concentration. Parallelism between the in-house control and the standard curve is excellent. However, inspection of the clinical data shows a very poor resolution between primary hyperparathyroid patients and normal controls. Furthermore, a positive correlation (+0.201) between normal serum PTH and total serum calcium is obtained, whereas the correlation ought to be negative.

Iso-Tex and Nichols kits. Both the Iso-Tex kits and the Nichols kits (Figures 1 and 2) gave apparently rational and rather similar results in our hands, with a coefficient of correlation between the two kits of 0.86 if dialysis patients are excluded and 0.96 if dialysis patients are included. Discrimination among the three groups is acceptable, especially if serum calcium concentrations are taken into account. Negative correlations between the normal serum PTH and calcium concentrations are -0.147 and -0.137 for the Iso-Tex and Nichols kits, respectively.

Values for the patients on dialysis were substantially higher with the Iso-Tex kit, 21.46 ± 21.87 μg/L, than those obtained with the Nichols kit, 6.47 ± 2.34 μg/L. It is evident from Figure 2 that many of the sera from patients on dialysis must be diluted if an absolute value is to be established with the Iso-Tex kit rather than merely reporting ">12 μg/L." The value of such absolute data for patients on dialysis is questionable since, as already mentioned, the measurement of the C-terminal fragment of PTH for these patients does not reflect glandular status uniquely but also clearance of the biologically inactive fragments.

Recovery of Iso-Tex standard added to the in-house control was 118%, and recovery of the Nichols standard added to the in-house control was 122% when assayed with their respective reagents. However, addition of either standard to the in-house control and assay with the other company's reagents yielded high recoveries: 142% for the Nichols standard assayed with Iso-Tex reagents and 167% for the Iso-Tex standard assayed with Nichols reagents.

The Scatchard plot for the Nichols kit is closest to the ideal linear model (Figure 3). Turnaround time is better with the Iso-Tex kit (Table 1). Scatchard plot data for the other three kits are not included. The Cambridge and the Immuno Nuclear PTH-II kits, which require stripping techniques, do not meet the necessary condition underlying Scatchard plot analysis, i.e., that the assay be an equilibrium RIA. The Immuno Nuclear C-terminal kit, although it does not require a stripping step, is based on a nonequilibrium incubation; in addition, its positive correlation between normal serum PTH and total serum calcium eliminated it from further consideration.

There is no substantial difference in cost of the assay as performed with either the Nichols or the Iso-Tex kits. Certainly either one or both should be considered by any laboratory about to undertake the assay of serum PTH concentrations. However, we add a word of caution: The interpretation of PTH data, even with the best of methods, is not always straightforward; concentrations of serum calcium must be taken into account, and additional information, such as nephrogenous cyclic AMP values, is often helpful (5). A discussion of the clinical implications of hypercalcemia and serum PTH to be published in this journal may be useful where interpretation is required (2).

Note added in proof: Currently (Nov-Dec 1981), Iso-Tex is experiencing problems with these kits, resulting in a Scatchard plot with an apparent affinity constant ($K_a$) of 0.66 × 10$^{-9}$ and an apparent antibody concentration of $q = 2.53 \times 10^{-11}$ mol/L as compared with a $K_a = 131 \times 10^{-9}$ and $q = 4.92 \times 10^{-9}$ mol/L for the kits used in the above report.

References