Clinical and Laboratory Evaluation of a Radioimmunoassay Kit for Measuring the Mid-Molecule Fraction of Parathyrin

Virginia M. Haver,¹ Alan D. Rinker, Benjamin M. Ko,² and Norbert W. Tietz

We report the results of a laboratory and clinical evaluation of a commercial kit procedure (Immuno Nuclear Corp.) for measuring the mid-region of the parathyrin molecule. The estimated dose at 50% binding averaged 130 pmol/L, and the minimum detectable concentration was 14 pmol/L. The within-assay CV was ≤6.6%, the between-assay CV ≤12.5%. Relative analytical recoveries of various parathyrin fragments averaged 93% (intact, amino acid residues 1–84), 100% (midregion, 44–68), <0.01% (N-terminal, 1–34), and <0.01% (C-terminal, 69–84). Correlation of results obtained for 59 patients’ samples with a radioimmunoassay for mid-region/C-terminal parathyrin performed by the Mayo Medical Laboratory yielded the equation INC = 1.16 (Mayo) − 1.94 pmol/L (r = 0.971). Clinical evaluation indicates that the INC results for parathyrin correlate with the diagnoses of patients at least as well as the results obtained with the Mayo assay; in some cases, the INC procedure better distinguishes hyperfunction from normal parathyroid function. The INC procedure can be easily performed in hospital laboratories, with a 4-h turnaround time for results.

Additional Keyphrases: hyperparathyroidism · parathyroid status

Differential diagnosis of calcium disorders can be difficult, but measurement of parathyrin (PTH) has been helpful in their clinical evaluation. Historically, antibodies used in the radioimmunoassay of PTH have been directed either to the amino(N)-terminal or to the carboxy(C)-terminal region of the molecule.

Antisera against the N-terminal detect intact PTH and any fragments that include the biologically active residues 1–34. However, N-terminal assays have not always been helpful in distinguishing patients with primary hyperparathyroidism from normal individuals (1). Non-PTH proteins may interfere with tracer binding in these assays, causing falsely increased PTH values. Furthermore, biologically active fragments are short-lived, and falsely low values thus may be measured if secretion of PTH is episodic (2).

In the past, antibodies used in C-terminal PTH assays have been specific only for residues 53–84 of the PTH molecule. These assays detect intact PTH, large PTH fragments containing the mid-region plus the C-terminal, and small fragments containing the C-terminal without the mid-region. Assays specific for these fragments, which are biologically inactive but long-lived (3), are more useful in detecting primary hyperparathyroidism than are N-terminal assays. In renal disorders, however, C-terminal assays give high values for PTH, because the kidney is the primary route of removal of the C-terminal fragments. In these cases, the N-terminal or intact PTH assay may provide better diagnostic information (4, 5).

Recently, a mid-region fragment of PTH has been found in the sera of patients with primary and secondary hyperparathyroidism but not in the sera of apparently healthy persons (6). Mallette et al. (7) suggested that an assay that detects this fragment as well as the larger PTH fragment containing the mid-region and C-terminal may more sensitively distinguish patients with primary and secondary hyperparathyroidism from normal individuals.

Several commercial kits with mid-region/C-terminal specificity are now available, some with synthetic human PTH fragments as standards, with antisera that demonstrate good reactivity toward specific fragments of human PTH. We have evaluated a mid-molecule PTH kit from Immuno Nuclear Corp. (INC), Stillwater, MN 55082, and compared patients’ results obtained by this procedure with those obtained by an intact/mid-region PTH assay performed by the Mayo Medical Laboratory, Rochester, MN 55055. These results were then correlated with the patients’ presentations and diagnoses obtained by retrospective chart review. We performed this evaluation to determine whether we could obtain for our laboratory a test that was simple, had a short turnaround time, and could be performed at less cost in-house than methods previously available from referral laboratories.

Materials and Methods

Sera were collected from hospitalized patients being evaluated for parathyroid dysfunction. After collection, the sera were divided into two aliquots: one was mailed, packed in solid CO₂, to the Mayo Medical Laboratory for analysis on a fee basis; the other was stored at −80 °C for in-house assay with the INC kit. The paired aliquots were analyzed within two weeks of each other.

The RIA kit from INC contained six standards of synthetic human PTH (residues 44–88), ranging in concentration from 31.2 to 1000 pmol/L. The antiserum supplied with the kit was specific for the 44–88 region of PTH. Enzymatically prepared beef PTH (37–84) labeled with I²125 was used for the tracer. Free tracer was separated from bound with a precipitating complex containing normal chicken serum, rabbit or goat anti-chicken serum, and polyethylene glycol (all supplied with the kit). Analyses were performed by technologists in our routine radioimmunoassay laboratory. Standard curves were generated using a four-parameter logistic method. The normal reference interval for PTH in adults, as established by INC, is 29–85 pmol/L.

The antibody in the radioimmunoassay performed at the Mayo Medical Laboratory is claimed to exhibit mid-region C-terminal specificity (8). However, according to the published data, the assay has relatively poor specificity for the mid-region (44–88) and C-terminal (53–84) fragments (see Table 1). For adults, the normal reference interval for PTH...
Table 1. Relative Reactivity of Two Different Antibodies with Intact PTH and PTH Fragments

<table>
<thead>
<tr>
<th>Human PTH fragment</th>
<th>Mayo GP-235*</th>
<th>INC C-121</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–84</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>1–34</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>44–68</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td>53–84</td>
<td>4.5</td>
<td>—</td>
</tr>
<tr>
<td>65–84</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data from Kao et al. (8). Manufacturer's kit package insert. Determined in authors' laboratory. Range of recoveries for at least five different concentrations of each fragment is shown in parentheses.

in the Mayo Medical Laboratory assay is 20–70 μL-Eq/mL. Given that 5.34 pg of human 1–84 PTH (MW = 9500) is equivalent to 0.48 μL-Eq of Arnaud standard (8), this reference range corresponds to 23–82 pmol/L.

Human intact PTH fragments used in analytical recovery studies were obtained from Calbiochem–Behring Corp., La Jolla, CA 92037 (mid-region fragment, 44–68, and N-terminal, 1–34) and from Peninsula Laboratories, Belmont, CA 94002 (intact PTH, consisting of residues 1–84, and C-terminal, 69–84).

Results

Analytical variables for the INC kit are described below. Comparable data for the Mayo Medical Laboratory assay were unavailable.

**Binding parameters.** The average binding parameters were as follows (n = 9): nonspecific binding = 2.6%; PTH (44–68) concentration at 50% maximal binding = 130 ± 14.3 pmol/L; slope = 1.062 ± 0.057. The sensitivity (minimum detectable concentration) of the assay, determined as 2 SD of 10 analyses of the zero standard, was 14.4 pmol/L. Figure 1 shows a typical standard curve.

**Analytical recovery and specificity.** Recovery and specificity were determined by duplicate analyses of aliquots of a human plasma pool to which known amounts of PTH fragments had been added in five different concentrations. The average relative recoveries are shown in Table 1. Our results are expressed as relative reactivity in order to directly compare the specificity of the INC antibody with that of the Mayo antibody as described by Kao et al. (8). It is clear from this comparison that the reactivities of the two antibodies with the individual PTH fragments are different. The ability of the INC antibody to recognize human mid-region parathyroid fragments was further demonstrated by preparing serial dilutions of a patient's specimen to obtain final concentrations ranging from 12 to 735 pmol/L. Excellent parallelism with the INC standard curve was demonstrated (see Figure 1).

**Precision.** Within-assay precision was determined by repeated analyses of pooled human sera. Between-assay precision was determined by using the control material supplied with the kit. The results are shown in Table 2.

**Analytical and clinical correlation.** Fifty-nine patients' specimens were analyzed by both the INC and Mayo Laboratory procedures (Figure 2). Linear regression analysis yielded the equation INC = 1.16 (Mayo) – 1.94 (r = 0.971). Clinical histories were obtained from examination of the patients' medical records. Of the 59 patients studied, 12 were suspected or proven cases of primary hyperparathyroidism, 22 had chronic renal failure, three were cases of
suspected malignancy, one suffered from Fanconi syndrome and one from sarcoidosis, and the remaining 20 were classified as having idiopathic hypercalcemia or other non-parathyroid illnesses.

For specimens with PTH values >150 pmol/L, the two methods yielded similar results, supporting a diagnosis of primary or secondary hyperparathyroidism. However, we found some discrepancies between results of the two methods when the PTH values were close to the upper limits of the reference ranges, as illustrated in Figure 3. The greatest discrepancies are seen at the bottom right and top left corner of Figure 3. Table 3 presents laboratory data and probable diagnoses for these patients.

Patients GC and WE had slightly increased PTH concentrations when their sera were assayed by the Mayo Laboratory procedure, but the results fell within the reference interval when the same sera were assayed with the INC kit. Two months prior to testing, patient GC had undergone a total parathyroidectomy, with an implant of part of the gland in the left forearm. Implants are usually not functional until after three to four months, and so the patient was hypocalcemic at the time. Thus, the lower PTH value obtained by the INC assay was more compatible with the clinical picture and the calcium concentration in serum. Patient WE had a history of hypercalcemia and renal stones. However, during several months of testing, values for total and ionized serum calcium and cyclic AMP were consistently normal, as was renal function. At this time, parathyroid dysfunction was ruled out on clinical grounds; thus, the INC value more closely agreed with the clinical presentation.

Patient AS suffered from malnutrition, malabsorption, and vitamin D deficiency, and an increased PTH concentration would be consistent with a condition of secondary hyperparathyroidism. Both methods showed an increase in PTH for this patient; however, the INC-measured increase was significantly greater relative to its reference range and more sensitively indicated the clinical problem.

Patient AB exhibited symptoms consistent with the presence of a kidney stone. Additional laboratory data revealed increased phosphate in the urine, a slight increase in serum chloride, and ionized calcium values at the upper reference limit. Clinically mild hyperparathyroidism was suspected, and the increased PTH concentration measured by the INC assay was consistent with the patient's clinical presentation.

Patients JO and PM are both young women suffering from eating disorders (bulimia and anorexia). Many patients with eating disorders have a low dietary intake of calcium and show evidence of osteoporosis (9); however, in both of these women, the values for total calcium in serum were within normal limits, and there was no evidence of osteoporosis. The slightly above-normal PTH values obtained by the INC assay might suggest a dysfunction of the parathyroid gland, but further studies are needed on these patients.

Patient DF presented very discrepant PTH values as well as a very complex clinical picture. He had a history of hypertension, hypercalcemia, renal stones, and multiple fractures resulting from physical activities. A roentgenogram of the cervical spine suggested demineralization. However, values for ionized calcium, serum and urinary phosphorus, and previous PTH values were always within normal limits. At this time his diagnosis remains unresolved.

![Graph](image)

**Figure 3.** Correlation of patients' PTH values, illustrating the results within and near the reference ranges for the INC and Mayo Medical Laboratory assays.

<table>
<thead>
<tr>
<th>Reference interval</th>
<th>PTH, pmol/L</th>
<th>C\textsubscript{\text{a}}, mg/L</th>
<th>C\textsubscript{\text{ox}}, mmol/L</th>
<th>P, mg/L</th>
<th>Probable diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Age (y/sex)</td>
<td>INC 29-85</td>
<td>Mayo 23-82</td>
<td>98-101</td>
<td>1.12-1.23</td>
</tr>
<tr>
<td>GC</td>
<td>62/F</td>
<td>33</td>
<td>85</td>
<td>76</td>
<td>—</td>
</tr>
<tr>
<td>WE</td>
<td>57/M</td>
<td>37</td>
<td>96</td>
<td>94</td>
<td>1.18</td>
</tr>
<tr>
<td>WE</td>
<td>57/M</td>
<td>64</td>
<td>82</td>
<td>94</td>
<td>1.22</td>
</tr>
<tr>
<td>AS</td>
<td>89/F</td>
<td>134</td>
<td>87</td>
<td>75</td>
<td>1.23</td>
</tr>
<tr>
<td>AB</td>
<td>35/F</td>
<td>88</td>
<td>47</td>
<td>94</td>
<td>1.18</td>
</tr>
<tr>
<td>JO</td>
<td>23/F</td>
<td>91</td>
<td>66</td>
<td>104</td>
<td>1.22</td>
</tr>
<tr>
<td>PM</td>
<td>23/F</td>
<td>91</td>
<td>44</td>
<td>91</td>
<td>1.22</td>
</tr>
<tr>
<td>DF</td>
<td>48/F</td>
<td>93</td>
<td>&lt;23</td>
<td>97</td>
<td>1.18</td>
</tr>
<tr>
<td>HB</td>
<td>69/F</td>
<td>88</td>
<td>89</td>
<td>91</td>
<td>1.23</td>
</tr>
<tr>
<td>WB</td>
<td>33/F</td>
<td>94</td>
<td>102</td>
<td>97</td>
<td>—</td>
</tr>
<tr>
<td>OLF</td>
<td>63/M</td>
<td>75</td>
<td>81</td>
<td>105</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Post-parathyroidectomy (2 mos)  
No parathyroid disease  
No parathyroid disease  
Malabsorption (secondary hyperparathyroidism)  
Hyperparathyroidism suspected  
Bulimia  
Bulimia/anorexia  
Fanconi syndrome/metabolic bone disease  
Uremic osteodystrophy  
Primary hyperparathyroidism
Both the INC and Mayo Laboratory PTH results for patients HB and WB were consistent with their clinical presentations. HB was diagnosed as having Fanconi syndrome with underlying metabolic bone disease. WB was diagnosed as having end-stage renal disease, and bone biopsy showed evidence of uremic osteodystrophy.

Patient OLF presented with a history of recurrent renal stones and a recent persistent increase in total and ionized calcium and chloride, and decreased values for bicarbonate. Although the PTH results of both assays were slightly less than the upper limit of normal, primary hyperparathyroidism was suspected and the left parathyroid glands were removed. Tissue examination indicated hyperplasia; values for ionized calcium returned to the normal reference interval before discharge from the hospital.

Discussion

The laboratory evaluation of the INC procedure indicated that the assay has good sensitivity, precision, and accuracy. Serial dilutions of a patient's specimen with abnormally high PTH concentration demonstrated excellent parallelism with the standard curve and indicated that the antibody has good recognition of human mid-terminal PTH fragments. The ability of the antibody to detect mid-region fragments was verified by analyzing a human plasma pool supplemented with known amounts of human PTH fragments.

An accurate and objective comparison of two different PTH methods is difficult, especially when a commercial method is compared with an in-house assay utilizing different antibodies, standards, and labeled reagents. Therefore, the clinical study was undertaken to confirm the validity of results obtained with the INC PTH radioimmunoassay.

The clinical study indicated that the INC assay performed at least as well as the Mayo Medical Laboratory assay, and in some cases showed an enhanced sensitivity in distinguishing hyperparathyroidism from normal parathyroid function. Although both radioimmunoassays are classified as mid-region/C-terminal assays, the antibody reactivities for various PTH fragments are quite different. Table 1 illustrates that the antiserum used in the Mayo Laboratory assay recognizes the mid-region fragment (residues 44–68) less than does the INC assay. If the mid-region fragment is truly an indicator of increased parathyroid secretory activity, then assays that best detect this fragment should show an enhanced sensitivity in distinguishing hyperparathyroidism from normal thyroid function.

We found no correlation between PTH measured with either the INC or Mayo assay and the identification of persons with renal osteodystrophy. Nevertheless, the INC assay shows great utility in detecting parathyroid hyperfunction and in discriminating hyperparathyroid patients from normals.

References