Summary and Explanation

Parathyroid hormone (parathyrin, PTH), a single-chain polypeptide (with a molecular mass of approximately 9,500 daltons) containing 84 amino acids, exerts significant influence in the maintenance of optimal calcium ion concentrations. PTH raises serum ionized calcium levels through direct action on bone and the kidneys: it increases the rate of calcium ion flow from bone to the extracellular fluid, and increases both the renal tubular reabsorption of ionized calcium and the renal excretion of phosphate. Long-term regulation of total body calcium by PTH occurs through its stimulation of vitamin D metabolism, which results in enhanced intestinal absorption of ionized calcium.¹

In healthy individuals, PTH is secreted in response to circulating calcium ion levels. Any dip below an individual's normal level triggers a pronounced increase in PTH secretion. Calcium levels returning to normal exert a negative feedback effect, thus inhibiting PTH secretion by the parathyroid glands.¹

PTH undergoes proteolysis to a lesser extent in the parathyroid glands but mostly peripherally – especially in the liver but also in the kidneys and bone – to yield N-terminal fragments and longer lived C-terminal and midregion fragments. The N-terminal fragment contains the region that confers bioactivity. C-terminal and N-terminal fragments are initially generated in equivalent amounts, but the N-terminal fragments disappear rapidly. The C-terminal fragment has a half-life of several hours. In renal failure, C-terminal fragment clearance by glomerular filtration is impaired, so that high levels are found. C-terminal assays (as well as midregion assays) are consequently likely to be especially unreliable in chronic renal failure, where increased PTH is typically just a reflection of impaired renal clearance.¹,²

For the intact hormone, the in vivo half-life is 2 to 5 minutes.³ Intact PTH clearance is accomplished by both peritubular uptake and glomerular filtration followed by reabsorption. In normal renal function, intact PTH is the greatest part of circulating PTH-like bioactivity⁴ and is present in the circulation at concentrations of 10⁻¹¹ to 10⁻¹² mol/L.²

In hypercalcemia due to primary hyperparathyroidism or to ectopic PTH production (pseudohyperparathyroidism), the majority of patients have elevated PTH levels. By contrast, in hypercalcemia due to malignancy or other causes, the concentration of PTH in circulation is typically low or within normal reference range limits. PTH levels are also characteristically high in secondary hyperparathyroidism – usually associated with renal failure – as a result of constant stimulation of the parathyroid gland by low calcium levels. Hypocalcemia accompanied by a low PTH level, on the other hand, is to be expected in hypoparathyroidism, either postsurgical or idiopathic.²,⁵,⁶

Immunoassays specific for various PTH fragments have been developed. Most rely on antisera specific for a discrete region: the C-terminal, N-terminal, or midmolecule. The antisera employed in such assays recognize not only the specific region, but similar fragments as well.¹,⁴

Recent assays for intact PTH have the necessary sensitivity for detecting circulating intact PTH in normals and for discriminating between normals and those with primary hyperparathyroidism. These assays also appear to discriminate better between primary hyperparathyroidism and hypercalcemia of malignancy compared with previous assays, and do so virtually without any significant overlap between these groups.⁴

Much improved clinical sensitivity is reported for PTH assays when dynamic reference intervals (based on a range of serum PTH values obtained by acute modification of serum calcium
concentrations in healthy subjects) are used, rather than a gaussian reference range (based on PTH values seen in normocalcemic individuals). Using an immunoradiometric assay for intact PTH and applying a dynamic reference range, Lepage, et al. obtained average clinical sensitivity of up to 100 percent with primary hyper- and hypoparathyroid samples. Moreover, only the intact PTH assay allowed complete separation between primary hyperparathyroid and nonparathyroidal hypercalcemic patients.⁷

**Principle of the Procedure**

IMMULITE 2000 Intact PTH is a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay.

**Incubation Cycles:** 1 × 60 minutes.

**Specimen Collection**

Collect blood by venipuncture into plastic serum tubes (with or without gel barrier), avoiding hemolysis. Serum should be separated from the cells as soon as possible.

Allow the specimens to clot at room temperature. Separate the serum from the cells, using a refrigerated centrifuge, if possible. PTH is only stable for 2 hours at room temperature.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

**Volume Required:** 50 µL serum (excluding dead space)

**Storage:** Samples are only stable for 2 hours at room temperature. Samples can be stored at 2–8°C for up to 8 hours after collection. For longer storage, aliquot and freeze up to 2 months at –20°C.

**Warnings and Precautions**

For *in vitro* diagnostic use.

**Reagents:** Store at 2–8°C. Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

**Materials Required**

**Chemiluminescent Substrate:** Allow substrate to equilibrate at room temperature (about 20 minutes) prior to opening bottle and dispensing. Avoid contamination and exposure to direct sunlight. Stable on board after opening at 15-28°C for 30 days, or until expiration date.
**Probe Wash (L2PWSM):** Add ~ 1500 mL of Type I deionized water to a clean labeled probe wash container. Pour the entire contents of one bottle of probe wash into the container and QS to 2000 mL with type 1 water. Stable at 15-28°C until the expiration date.

**Probe Cleaning Kit (L2KPM):** 100mL of solution in a brown plastic bottle. Stable at 15-28°C until the expiration date.

**Reaction Tubes (LRXT) (disposable)**
Components are a matched set. Labels on the inside box are needed for the assay.

**Intact PTH Bead Pack (L2PP12)**
With barcode. 200 beads, coated with affinity-purified murine monoclonal anti-PTH (44-84) antibody. Stable at 2–8°C until expiration date.

**Intact PTH Reagent Wedge (L2PPA2)**
With barcode. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to affinity-purified goat polyclonal anti-PTH (1-34) in buffer, with preservative. Stable at 2–8°C until expiration date.

**Intact PTH Adjustors (LPHL, LPHH)**
Two vials (Low and High) of lyophilized, synthetic human intact PTH in a buffered matrix. Reconstitute each vial with 2.0 mL deionized type 1 water. Mix by gentle, intermittent swirling. Use only freshly reconstituted Adjustors for each assay. Do not freeze.

**Intact PTH Sample Diluent (L2PHZ)**
For on-board dilution of high samples. 25 mL of concentrated (ready-to-use) PTH-free buffer matrix. Stable at 2–8°C for 30 days after opening.

**DPC Immulite Intact PTH controls levels 1 and 2**
Reconstitute each bottle with 2.0 mL of deionized type 1 water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. After reconstitution, aliquot 0.4 mL into 13x100 false bottom tubes labeled with a control barcode. Freeze aliquots at -20°C. Stable for 4 weeks.

### Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.


**Recommended Adjustment Interval:** 4 weeks.
Expected Values

A reference range study was conducted on matched EDTA plasma and serum samples from 88 apparently healthy volunteers, collected into BD plastic Vacutainer™ tubes. The matched samples were analyzed on IMMULITE/IMMULITE 1000 Intact PTH and on IMMULITE 2000 Intact PTH assays. Analysis of the data indicated no statistically significant difference between platforms, although there was a clear difference in reference ranges between EDTA plasma and serum. The reference ranges suggested by this study for IMMULITE/IMMULITE 1000 Intact PTH and IMMULITE 2000 Intact PTH for both EDTA plasma and serum are shown in the following table.

The reference range for serum is in good agreement with a previous multi-site reference range study, conducted with IMMULITE 2000 Intact PTH on 255 serum samples from apparently healthy subjects. This population yielded a median of 32 ng/L. The reference range suggested by this study is:

Reference range: 12 – 65 ng/L (1.3 – 6.8 pmol/L).

Limitations

The assay is intended strictly as an aid in the differential diagnosis of hypercalcemia and hypocalcemia, not for the diagnosis or management of malignancy.

Because of the physiological relationship between circulating calcium and PTH, it is always important to interpret PTH results in light of total or ionized calcium levels. The finding of a persistently high-normal calcium accompanied by a high-normal PTH (alternatively, a low-normal calcium accompanied by a low-normal PTH) warrants further investigation; for the PTH, though itself within normal limits, may still be inappropriately high (or inappropriately low) relative to the circulating calcium level.

Indices of renal function, e.g. creatinine levels; measurement of albumin, as an adjunct to measurement of total calcium levels; determinations of phosphorus, chloride, nephrogenous cyclic AMP and possibly calcitonin, may also (in certain circumstances) aid in the interpretation of PTH and calcium results. It should also be remembered that hypercalcemia and hypocalcemia may be secondary to disordered vitamin D metabolism.

Lipemic, hemolyzed, icteric or grossly contaminated samples may give erroneous results.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with in vitro immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1998;34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.
Performance Data

**Conversion Factor:**
ng/L × 0.1053 → pmol/L

**Calibration Range:** Up to 2,500 ng/L (263 pmol/L).

**Analytical Sensitivity:** 3.0 ng/L (0.3 pmol/L).

**High-Dose Hook Effect:** None up to 500,000 ng/L (50,000 pmol/L).

**Intraassay Precision:** Statistics were calculated for samples from the results of 20 replicates in a single run.

### Intraassay Precision (ng/L)

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>sd</th>
<th>cv</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>4.1</td>
<td>5.7%</td>
</tr>
<tr>
<td>2</td>
<td>258</td>
<td>11</td>
<td>4.3%</td>
</tr>
<tr>
<td>3</td>
<td>662</td>
<td>28</td>
<td>4.2%</td>
</tr>
</tbody>
</table>

**Interassay Precision:** Statistics were calculated for samples assayed in 10 different runs.

### Interassay Precision

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>sd</th>
<th>cv</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>3.4</td>
<td>6.3%</td>
</tr>
<tr>
<td>2</td>
<td>387</td>
<td>34</td>
<td>8.8%</td>
</tr>
</tbody>
</table>

**Specificity:** The antibody is highly specific for intact PTH.

### Specificity

<table>
<thead>
<tr>
<th>Compound,</th>
<th>ng/L Added</th>
<th>% Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (1-34)</td>
<td>300</td>
<td>ND</td>
</tr>
<tr>
<td>PTH (1-44)</td>
<td>100,000</td>
<td>ND</td>
</tr>
<tr>
<td>PTH (44-68)</td>
<td>100,000</td>
<td>ND</td>
</tr>
<tr>
<td>PTH (53-84)</td>
<td>100,000</td>
<td>ND</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>10,000</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not detectable.
Antibodies used in the kit were purified by affinity chromatography to achieve specificity for well-defined regions of the intact PTH molecule. The antibodies immobilized to the solid phase (coated bead) are specific for the C-terminal region (44-84) and have no detectable crossreactivity to the N-terminal region (1-34). Conversely, the enzyme-labeled antibody recognizes only the N-terminal region, and has no detectable crossreactivity to C-terminal or midmolecule regions. Accordingly, the assay, which requires binding by both enzyme-labeled and solid-phase antibodies, is able to recognize only intact PTH and very large PTH fragments that are nearly as long as intact PTH itself. One such fragment, PTH 7–84, exhibits significant crossreactivity (44.8%) in the IMMULITE 2000 PTH assay.

**Bilirubin:** Presence of conjugated and unconjugated bilirubin in concentrations up to 200 mg/L may cause a depression of values.

**Hemolysis:** Presence of hemoglobin in concentrations up to 513 mg/dL may cause a depression of values.

**Lipemia:** Presence of triglycerides in concentrations up to 3,000 mg/dL may cause a depression of values.

If there is an interferant (hemolysis, lipemia and icterus), run the sample undiluted and send the corresponding ETC Code:

- **TUR** “Specimen turbid, result may be invalid”
- **ICTRQ** “Specimen icteric, result may be invalid”
- **HEMRQ** “Specimen hemolyzed, result may be invalid”

**Reporting values:** Values below 3 ng/L are reported as <3. Values above 2,500 ng/L are reported as >2,500 unless they are part of a catheterization study (see section on performing dilutions below).

<table>
<thead>
<tr>
<th></th>
<th>Regular Sample</th>
<th>Sample from catheterization study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMR (Analytical Measuring Range)</strong></td>
<td>3 ng/L to 2,500 ng/L</td>
<td>3 ng/L to 2,500 ng/L</td>
</tr>
<tr>
<td><strong>Reportable Range</strong></td>
<td>3 ng/L to 2,500 ng/L</td>
<td>3 ng/L to (dilute until answer is achieved)</td>
</tr>
</tbody>
</table>

**NOTE:** Dilutions are performed only on catheterization study samples that are >2,500 ng/L. Catheterization studies can be identified based on multiple samples submitted on one patient with the same collection date and time. For these study samples, dilutions are performed until an answer is achieved as the clinician needs to know which sample has the highest value.

**Performing dilutions**

**On-Board Dilutions:**

- Place the PTH sample diluent labeled with the appropriate barcode into a sample rack
- Place the sample(s) needed for dilution into a sample rack
- Press Run
After the samples have been scanned, go into the Worklist screen
Select Next. The first patient sample accession # should appear.
Select Test.
Select test iPT and press OK
Select Dilution
Select iPT
Select the appropriate dilution factor (e.g. x10).
At this point, you may do another dilution (e.g. x20) on the same sample by starting again at “Select test”.
Select Accept Patient.

Note: the printed value will be corrected for the dilution factor. You do not need to multiply the printed value.

Note: If the PTH result is still too high after performing a x100 dilution, the sample must be diluted manually with Immulite 2000 PTH Sample Diluent.

Manual Dilutions:

Prior to use as a manual diluent, the Immulite PTH Sample Diluent must be diluted 1 part diluent to 1.5 parts Type 1 deionized water. To program manual dilutions on the Immulite 2000 perform the following steps:

- Place the manually diluted sample (in a false bottom tube without a barcode) into an Immulite sample rack.
- Push RUN.
- A “Report” saying “There is 1 tube in Sample Rack ___ with no barcode” will appear on the instrument screen.
- Select CLOSE.
- Select WORKLIST.
- Select ASSIGN TUBE POSITION.
- Select the rack in which your dilution is located.
- Select the position in the rack where your dilution is located.
- Select OK.
- Enter the accession # times the dilution factor (e.g. 12345 x 5).
- Select TEST.
- Select iPT.
- Select OK.
- Select MANUAL DILUTION.
- Enter the dilution factor you used for this sample.
- Select ACCEPT PATIENT.
The printed value will be corrected for the dilution factor. You do not need to multiply the printed value by the dilution factor.

References


4) Measuring the PTH level. Lancet 1988 (Jan 16);94-5.


8) Logue FC, Fraser WD, O'Reilly DStJ, Beastall GH. The circadian rhythm of intact parathyroid hormone (1-84) and nephrogenous cyclic adenosine monophosphate in normal men. J Endocrinol 1989;121:R1-R3.


21) Immulite 2000 Intact PTH reagent insert