Intact PTH Assay

Immunoradiometric (IRMA) Assay
(Coated Bead-Technology)
For the quantitative determination of human intact PTH.
For in vitro diagnostic use only

INTENDED USE
The Scantibodies Intact PTH Coated Bead Kit has been designed for the quantitative determination of human intact parathyroid hormone (PTH) in serum.

PHYSIOLOGY
The intact PTH peptide (1-84) is secreted by parathyroid glands under the control of the concentration of ionized calcium, vitamin D and magnesium in blood. PTH acts with respect to calcium on the kidneys and the skeleton\(^1,2\). PTH binds to receptors, which stimulate adenylylate-cyclase to produce cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP)\(^3\). PTH is metabolized in the kidney by cleaving the peptide between positions 33 and 34\(^6,7\). The N-terminal fragment (1-33) seems to be further cleaved. Biologically inactive fragments with molecular weights of 4000-7000 circulate with a half-life of 30 minutes in healthy persons\(^4,5\). cAMP or other PTH dependent processed metabolites (e.g. Hypophosphatemia) stimulate the renal hydroxylation of 25-(OH) vitamin D to 1,25-(OH)\(_2\) vitamin D. This vitamin D metabolite stimulates calcium absorption by the small intestine. Severe vitamin D deficiency results in an enhanced secretion of PTH compared to the secretion of calcium. Hypomagnesemia in the primary stage stimulates hypocalcemia. Severe hypomagnesemia results in the reduced secretion of PTH. The biological activity of PTH resides in the first 34 amino acids of the N-terminal portion of the molecule.\(^12,13,18,19\)

Primary and secondary hyperparathyroidism, kidney insufficiency, malabsorption-syndrome and pseudo-hypoparathyroidism result in elevated concentrations of PTH\(^14,15,16\). Decreased concentrations of PTH coincide with high doses of vitamin-D, milk-alkali-syndrome, Morbus Boeck, hyperthyreosis, ingestion of thiadiz and hypercalcaemia of malignancy. PTH concentration is also decreased with absorptive hypercalciuria and hypopara thyroidism. Due to a pronounced nocturnal rise in intact PTH, sample collection times must be considered to distinguish between normal and mild hyperparathyroidism patients.

PRINCIPLE OF PROCEDURE
Scantibodies Intact PTH Kit is an immunoradiometric (IRMA) assay utilizing a polyclonal 1-84 PTH antibody with a tendency to bind in the N terminal region of 1-84 PTH (Label Antibody), and a polyclonal 1-84 PTH antibody with a tendency to bind in the C terminal region of 1-84 PTH (Capture Antibody). As both the Label and Capture Antibodies bind both 1-84 PTH and truncated fragments of PTH the use of these antibodies guarantees that both 1-84 PTH and truncated forms of PTH are detected. The Label Antibody is labeled with \(^{125}\)I. The Capture Antibody is fixed to the beads. The Intact PTH (meaning 1-84 PTH and truncated forms of PTH) in patient samples is bound both to the beads and the Label Antibody. Simple wash steps reduce the non-specific binding (NSB) to a minimum for increased precision at the low end of the calibration curve. The concentration of intact PTH is directly proportional to the radioactivity bound to the beads after separation. The concentration of PTH in unknown patient samples and controls is determined by interpolation using a calibration curve.\(^12,17\)

REAGENTS
The Scantibodies Intact PTH Coated Bead Kit contains sufficient reagents for 100 single determinations. The kit is stable at 2 – 8°C until the stated expiration date.

Scantibodies PTH Calibrators
One set of calibrators consists of six vials containing lyophilized human serum with nominal synthetic intact PTH concentrations. The lyophilized calibrators are prepared in stabilized human serum containing sodium azide 0.1% (w/v). The intact PTH concentrations are declared on the vial label.

Scantibodies PTH Controls
One set of controls consists of two vials containing synthetic human intact PTH in lyophilized human serum with 0.1% (w/v) sodium azide. The intact PTH concentrations are declared on the vial label.

Scantibodies PTH \(^{125}\)I-Anti PTH (1-34) Tracer
One set of tracer consists of two bottles of \(^{125}\)I-anti PTH (1-34). Each bottle contains polyclonal goat anti PTH (1-34) which is labeled with \(^{125}\)I and dissolved in 5 ml phosphate buffered saline with sodium azide 0.1% (w/v) and protein stabilizers. The maximum radioactivity in a bottle is <370 kBq (<10 \(\mu\)Ci ).

Scantibodies PTH Antibody (39-84) Coated Beads
One jar contains 100 polystyrene beads (8 mm
diameter) plus desiccant. The beads are coated with polyclonal goat anti-PTH (39-84). The desiccant contains silica.

**Scantibodies PTH Wash Concentrate**

One bottle contains 30 ml of a 30 fold concentrate of phosphate buffered saline with sodium azide 1.5% (w/v) and detergent.

**PREPARATION AND STORAGE OF REAGENTS**

**Scantibodies PTH Calibrators**

The Scantibodies Laboratory, Inc. Intact PTH Coated Bead Diagnostic Kit contains the PTH standards prepared analytically on a mass basis from purified synthetic intact PTH (1-84). These standards are further evaluated against "primary standards" which are stored at −70° C to maintain calibration.

Reconstitute the zero calibrator with 5 ml of distilled or deionized water. Reconstitute the remaining calibrators with 2 ml of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted calibrators within 1 hour. Store the unused portion of the calibrators below -20° C until the stated expiration date. Do not store the calibrators at room temperature for more than one hour at any given time. Do not thaw any calibrator vial more than two times. Do not use calibrators that exhibit precipitation or unusual color.

**Scantibodies PTH Controls**

Reconstitute the vials of controls with 2 ml of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted controls within 1 hour. Store the unused portion of the controls below -20° C until the stated expiration date. Do not store the controls at room temperature for more than one hour at any given time. Do not thaw any control vial more than two times. Do not use controls that exhibit precipitation or unusual color.

**Scantibodies PTH Antibody (39-84) Coated Beads**

The antibody coated beads are ready to use. Store the beads at 2 – 8° C until the stated expiration date. Allow the beads to equilibrate to ambient temperature prior to opening the jar. Tightly close the jar immediately after removing the required number of beads.

**Scantibodies PTH Wash Concentrate**

Dilute and mix well the 30 ml of wash concentrate with 870 ml of distilled or deionized water (1:30). Store the diluted wash solution at room temperature (18 - 25° C) until the stated expiration date. Do not use wash solution that shows precipitation.

**WARNINGS AND PRECAUTIONS FOR USERS**

**Use of The Assay**

The reagents are for in vitro diagnostic use only.

**Human Serum Caution**

The human serum in this kit has been prepared from human donors and it has been tested by FDA approved immunoassays and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Anti HIV I/II and Anti HCV. However, it is recommended to consider the calibrators and controls as a potential biohazard and handle them with the same precautions as applied to any untested patient sample.

**Radioactivity Warning**

This radioactive material may be received, acquired, possessed, or used only by physicians, clinical laboratories, or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority.

All radioactive materials must be disposed of according to the regulations (regulations differ from country to country) and guidelines of the agencies with jurisdiction over the laboratory. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used. Storage of radioactive materials should be limited to specifically designated and appropriately secured areas. Access to radioactive materials should be limited to authorized and trained personnel only. Do not pipette radioactive solutions by mouth. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves. Radioactive materials must be stored in designated areas in their original containers or in containers providing equivalent radiation protection. A record of disposal of all radioactive materials must be kept. Immediately remove spilled solutions and decontaminate contaminated devices. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.

**Sodium Azide (NaN₃) Warning**

Some reagents in the Scantibodies Intact PTH assay contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush the drain with a large volume of water to prevent azide build-up. Avoid direct contact with skin and mucous membranes.

**SAMPLE PREPARATION AND STORAGE**

**Specimen Collection**

The determination of human PTH must be made on serum. Four hundred microliters of serum are required to assay one sample in duplicate. To obtain serum, collect blood by venipuncture. Allow the blood to clot at room temperature. Centrifuge the sample and separate the serum from the cells. Sera should be stored at -20° C or lower. Avoid repeated freezing and thawing of sera. Do not use patient
samples which have been frozen and thawed more than two times.

**Dilution of Patient Samples**
Dilute serum samples with PTH concentrations greater than the highest calibrator with Scantibodies Intact PTH Zero Calibrator before assay. The dilution factor is applied to the diluted sample assay result in order to determine the PTH concentration in the undiluted sample.

**Quality Control**
Two levels of controls are provided with each assay kit. The values assigned to these controls are printed on the container label. The control value should fall within the specified range when tested in the same manner as the unknowns. Controls should be included in each assay. If the control values do not meet the established range, the assay may be invalid and should be repeated.

**ASSAY PROCEDURE**

**Materials Provided**
The Scantibodies Intact PTH Coated Bead Kit (Part No. 3KG551) is supplied with the following:

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scantibodies PTH Calibrators</td>
<td>6 vials</td>
</tr>
<tr>
<td>Part Nos. 3CA001, 3CB001, 3CC001, 3CD001, 3CE001, 3CF001</td>
<td></td>
</tr>
<tr>
<td>Scantibodies PTH Controls</td>
<td>2 vials</td>
</tr>
<tr>
<td>Part Nos. 3CA002, 3CB002</td>
<td></td>
</tr>
<tr>
<td>Scantibodies PTH Antibody (39-84) Coated Beads Part No. 3KB001</td>
<td>1 jar of 100 beads</td>
</tr>
<tr>
<td>Scantibodies PTH 125I-anti PTH (1-34) Antibody Part No. 3KL001</td>
<td>2 vials</td>
</tr>
<tr>
<td>Scantibodies PTH Wash Concentrate Part No. 3KW001</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Directional Insert Part No. 3KI088</td>
<td>1 insert</td>
</tr>
</tbody>
</table>

**Materials And Equipment Required But Not Provided:**
Distilled or deionized water
Round-bottomed polypropylene or polystyrene test tubes (12 x 55, 12 x 75, 12 x 70 mm or equivalent)
Pipettor with disposable tips: 0.2 ml
Repeating dispenser: 0.1 ml
Bead dispenser or plastic tweezers
Wash station
Vortex mixer
Gamma counter calibrated to detect 125I

**Preparation for Assay**
For each assay, prepare the following groups of tubes and place them in a test tube rack (double determination):
- 2 total count tubes (optional for QC)
- 2 Bo tubes (NSB)
- 2 tubes for each calibrator concentration
- 2 tubes for each control concentration
- 2 tubes for each patient sample

**Pipetting and Incubation Steps**
1. Pipette 0.2 ml of calibrators, samples and controls into the corresponding tubes.
2. Pipette 0.1 ml of Scantibodies Intact PTH 125I-anti PTH (1-34) into each tube.
3. Gently vortex all tubes.
4. Dispense one antibody coated bead into each tube except for the total count tubes. To add the beads, tilt the test tube rack to approximately a 30 degree angle to prevent splashing.
5. Seal the tubes and incubate them for 18 - 24 hours at room temperature (18 - 28° C).
6. Aspirate the supernatant from each tube except for the total count tubes. Wash the beads 3 times with 2 ml of diluted wash solution. After each addition of diluted wash solution aspirate all of the wash solution.
7. Count each tube for at least 1 minute in a gamma counter calibrated to detect 125I. The total count tube should contain approximately 300,000 CPM (assuming the counter has an efficiency of 70% - 80%) when freshly iodinated 125I-anti PTH (1-34) is used. The total activity of the tracer decreases according to the half life of 125I.

**PIPETTING GUIDE**

<table>
<thead>
<tr>
<th>Additive To Tube</th>
<th>Total Count Tubes</th>
<th>Bo Tubes</th>
<th>Calibrator Tubes</th>
<th>Control Tubes</th>
<th>Sample Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator</td>
<td>-</td>
<td>200 µl</td>
<td>200 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200 µl</td>
<td>200 µl</td>
</tr>
<tr>
<td>125I anti PTH (1-34)</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Beads</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Vortex mix all tubes, except for the TC tubes. Incubate tubes for 18 - 24 hours at room temperature (18 - 28° C).*

Aspirate the supernatant from all of the tubes except the total count tubes. Wash all beads by adding 2 ml of diluted wash solution to all tubes except the total count tubes and aspirating the wash solution. Repeat this wash step two more times for a total of three times.

Count each tube for at least 1 minute in a gamma counter.

**PROCEDURAL COMMENTS**

**Known Interferences:**
Grossly hemolyzed or lipemic samples.
Samples from patients receiving radioisotopes for ...
diagnostic or therapeutic purposes. Contamination of the sample or assay tube with $^{125}$I or other radioisotopes.

**Reagents from different lot numbers must not be interchanged.**

The patient sample or calibrator and the $^{125}$I-anti PTH (1-34) should be pipetted carefully into the bottom one-fourth of the assay tube. This is to avoid losing liquid on the surface of the tube as the liquid runs down the tube. The washing step is an important step in the assay procedure. Accurate dispensing of the wash solution and complete aspiration of the tube contents is essential to achieving assay sensitivity, low background and assay precision.

Do not handle beads with your hands. Use a plastic forcep or equivalent.

Standards must be frozen immediately after use and may only be thawed and reused a maximum of two times provided acceptable control results are obtained.

It is recommended that calibrators and patient samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.

When adding the beads to the tubes, tilt the test tube rack to a 30 degree angle to avoid splashing.

Avoid sample to sample contamination by using a new pipette tip for each sample.

**CALCULATION OF RESULTS**

**Calculation**

1. Calculate the average CPM for each double determination.
   \[ (\text{CPM}_1 + \text{CPM}_2) \div 2 = \text{Average CPM} \]
2. Subtract the average CPM of the zero calibrator tubes from the CPM's from all other tubes in order to obtain the corrected CPM for each tube.
3. Corrected CPM = average CPM of duplicate samples - average CPM of duplicate zero calibrators.
4. Draw the calibration curve by plotting the average corrected CPM from each duplicate calibrator level (ordinate) against the respective concentration declared on the calibrator vial (absolute) using log-log graph paper. Obtain sample concentrations by interpolation of average sample CPM on the calibration curve.
5. If samples were run with dilution, multiply the diluted sample assay results from the curve by the appropriate dilution factors to obtain the undiluted sample assay results.
6. Values less than lowest std:--
   \[ \text{Value of unknown} = \frac{\text{Corrected CPM (unknown)} \times \text{Value of lowest standard}(\text{pg/mL})}{\text{Corrected CPM (lowest std)}} \]

**Sample Data**

<table>
<thead>
<tr>
<th>Tube</th>
<th>CPM</th>
<th>Avg. CPM</th>
<th>Corrected CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Activity</td>
<td>339069</td>
<td>336321</td>
<td>337695</td>
</tr>
<tr>
<td>0 pg/ml</td>
<td>800</td>
<td>751</td>
<td>776</td>
</tr>
<tr>
<td>12 pg/ml</td>
<td>2381</td>
<td>2348</td>
<td>2365</td>
</tr>
<tr>
<td>47 pg/ml</td>
<td>5552</td>
<td>5450</td>
<td>5501</td>
</tr>
<tr>
<td>175 pg/ml</td>
<td>14904</td>
<td>14654</td>
<td>14779</td>
</tr>
<tr>
<td>475 pg/ml</td>
<td>41482</td>
<td>41553</td>
<td>41518</td>
</tr>
<tr>
<td>1600 pg/ml</td>
<td>108811</td>
<td>104611</td>
<td>106711</td>
</tr>
</tbody>
</table>

**NOTE:** The data presented are for demonstration purposes only and must not be used in place of data generated at the time of the assay.

**REPRESENTATIVE STANDARD CURVE**

Automated data reduction can also be used to construct the Scantibodies Intact PTH calibration curve. To program automated data reduction systems or to adapt an existing program, consult the data processor manufacturer or the programmer.

**LIMITATIONS OF THE PROCEDURE**

For diagnostic purposes PTH values should be used in addition to other diagnostic data and clinical information available to the physician.

The assay procedure must be followed exactly; careful technique must be used to obtain valid results. Any modification of the assay procedure is likely to alter the results.

Grossly hemolyzed, lipemic or icteric samples are likely to give non valid results.

The highest concentration of PTH measurable without sample dilution is the concentration of the highest calibrator. The lowest level measurable is approximately 1...
pg/ml.

**High Dose Hook Response**

The high dose hook response of the Scantibodies Laboratory, Inc. Intact PTH Coated Bead Diagnostic Kit was determined as 100,000 pg/ml of Intact PTH. Samples greater than the highest standard (approximately 1500 pg/ml) and up to 100,000 pg/ml intact PTH will read CPM values greater than that of the highest standard.

**EXPECTED VALUES**

It is recommended that each laboratory establish its own range of normal values. The values given are only indicative and may vary from other published data22.

**PERFORMANCE CHARACTERISTICS**

**Analytical Recovery**

Different samples with low concentrations of PTH were spiked with 2 different amounts of PTH. The % recovery was determined following assay of the spiked samples.

<table>
<thead>
<tr>
<th>Sample value (pg/ml)</th>
<th>Added PTH(pg/ml)</th>
<th>Expected value (pg/ml)</th>
<th>Measured value (pg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>101</td>
</tr>
<tr>
<td>150</td>
<td>45</td>
<td>74</td>
<td>75</td>
<td>112</td>
</tr>
<tr>
<td>34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>150</td>
<td>45</td>
<td>79</td>
<td>76</td>
<td>104</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>107</td>
</tr>
<tr>
<td>150</td>
<td>45</td>
<td>70</td>
<td>75</td>
<td>110</td>
</tr>
</tbody>
</table>

**Linearity of Patient Sample Dilutions**

Different samples with high concentrations of PTH were diluted in a sera with the zero standard. The % recovery was determined following assay of the diluted samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added PTH (pg/ml)</th>
<th>Expected value (pg/ml)</th>
<th>Measured value (pg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>581</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>291</td>
<td>303</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>145</td>
<td>143</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>73</td>
<td>82</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>325</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>163</td>
<td>151</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>81</td>
<td>82</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>41</td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>

**Precision**

Intra-assay coefficient of variation was evaluated by performing 10 replicate determinations on two serum pools in the same assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>2.2</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>271</td>
<td>12</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Inter-assay coefficient of variation was evaluated by performing 10 different assays on two serum pools over a 2 day period.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>3.2</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>270</td>
<td>19</td>
<td>7.0</td>
</tr>
</tbody>
</table>

**Sensitivity**

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero. This sensitivity was determined by assaying the zero calibrator 20 times in the same assay. The detection limit, as determined by the 95% confidence limit, is approximately 1 pg/ml.

**Specificity**

The specificity of the Scantibodies Laboratory, Inc. Intact PTH Coated Bead Diagnostic Kit was determined by spiking a solution of intact PTH (concentration: 82 pg/ml) with PTH fragments in the concentrations indicated.

<table>
<thead>
<tr>
<th>PTH FRAGMENT</th>
<th>CONCENTRATION</th>
<th>% CROSS REACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 34</td>
<td>400 pg/ml</td>
<td>Undetectable (&lt;0.1%)</td>
</tr>
<tr>
<td>39 - 68</td>
<td>100,000 pg/ml</td>
<td>Undetectable (&lt;0.1%)</td>
</tr>
<tr>
<td>53 - 84</td>
<td>100,000 pg/ml</td>
<td>Undetectable (&lt;0.1%)</td>
</tr>
<tr>
<td>44 - 68</td>
<td>100,000 pg/ml</td>
<td>Undetectable (&lt;0.1%)</td>
</tr>
<tr>
<td>39 - 84</td>
<td>100,000 pg/ml</td>
<td>Undetectable (&lt;0.1%)</td>
</tr>
</tbody>
</table>

The Scantibodies Intact PTH assay is not affected by high levels of C-terminal fragment PTH found in renal failure patients. The following dilution study demonstrates the accuracy of the assay when a renal failure patient's serum was diluted in a serum with low concentration of PTH. The % recovery was determined from the expected versus the observed assay results. Had the C-terminal fragment in the following serum caused an interference, the recovery would have been lower than 100%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added PTH (pg/ml)</th>
<th>Expected value (pg/ml)</th>
<th>Measured value (pg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>581</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>1:8</td>
<td>73</td>
<td>82</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>325</td>
<td>-</td>
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<tr>
<td></td>
<td>1:2</td>
<td>163</td>
<td>151</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>81</td>
<td>82</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>41</td>
<td>41</td>
<td>100</td>
</tr>
</tbody>
</table>
have been greater than 100% as the undiluted value would be suppressed by fragment binding to the solid phase.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Expected</th>
<th>Observed</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>undiluted</td>
<td>400</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>1:2</td>
<td>200</td>
<td>212</td>
<td>108</td>
</tr>
<tr>
<td>1:4</td>
<td>100</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>1:8</td>
<td>50</td>
<td>49</td>
<td>97</td>
</tr>
</tbody>
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Correlation

There is a high degree of correlation between the serum PTH levels of sample measured in duplicate by the Nichols Institute Diagnostics Coated Bead Kit and those levels measured by the Scantibodies Laboratory, Inc. (SLI) Intact PTH Coated Bead Diagnostic Kit. A correlation coefficient ($r$) of 0.99 ($n=49$) was obtained with a slope of 1.19 and intercept of -6.55 where $X$ represents the Nichols Diagnostics data and $Y$ represents the SLI data. Calculations were made with samples ranging from 5.6 - 828.2 pg/ml.

**Chemical Characterization:**

1) Antibodies coated on to polystyrene Beads (or tubes).
2) Radioactive Isotope containing Iodine-125 with radioactivity $<10$ µCi and Sodium Azide @ 0.1%.
3) Calibrators & Controls – Human Serum containing Sodium Azide @ 0.1%
4) Wash Concentrate containing sodium azide @ 1.5%.

**Hazardous ingredients:**

Radioactive Isotope (Iodine-125) @ $<10$ µCi/Vial (<370 kBq)
CAS Number: 7553-56-2
Symbols: Harmful Xn
R-phrases: R22, R52/53
S-phrases: S28, S45, S53, S60, S61
Sodium Azide @ 0.1%
CAS Number: 026628-22-8
Symbols: Harmful Xn
R-phrases: R22, R31, R52/53
S-phrases: S28, S45, S53, S60, S61
Sodium Azide @ 1.5%
CAS Number: 026628-22-8
Symbols: Very Toxic T+; N
R-phrases: R28, R32, R50/53
S-phrases: S28, S45, S53, S60, S61

**PTH BIBLIOGRAPHY**

3. Flueck, J., DiBella, F., Kehrawal, J., and Arnaud, C. "Immunoheterogeneity of PTH in Venous Effluent Serum from Hyperfunctioning Parathyroid..."