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(54) **METHODS FOR DIFFERENTIATING AND MONITORING PARATHYROID AND BONE STATUS RELATED DISEASES**

cation No. 09/231,422, filed on Jan. 14, 1999, now Pat. No. 6,689,566.

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(57) **ABSTRACT**

The present invention relates to novel methods and devices for differentiating in a patient parathyroid diseases, such as hyperparathyroidism and related bone diseases, from normal or non-disease states. One detects whole or non-fragmented (1 to 84) parathyroid hormone in a biological sample and also a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist. By either comparing values or using independently the value of either the large non-whole parathyroid hormone peptide fragment, the whole parathyroid hormone, or the combination of these values one is able to differentiate parathyroid and bone related disease states, as well as differentiate such states from normal states.

**Related U.S. Application Data**

(63) Continuation of application No. 10/760,091, filed on Jan. 16, 2004, which is a continuation of application No. 09/344,639, filed on Jun. 26, 1999, now Pat. No. 6,743,590, which is a continuation-in-part of appli-

# FIG. 1

## Whole Human PTH (1-84)

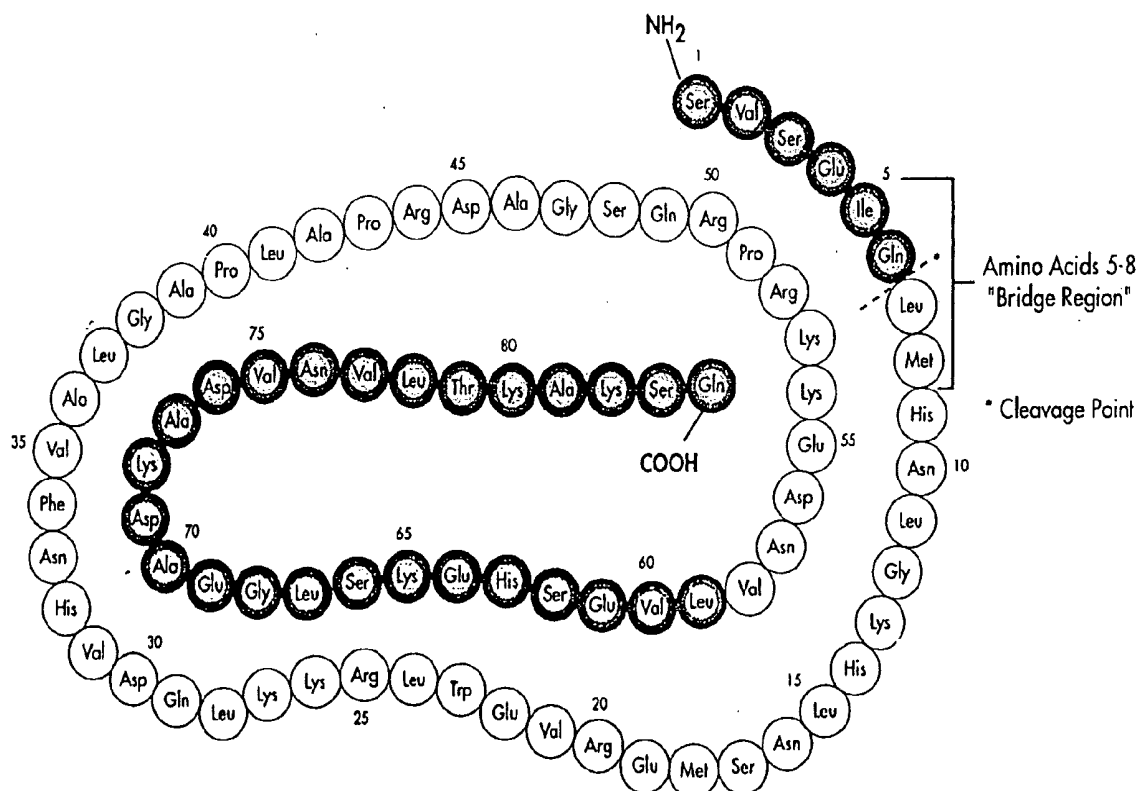


FIG. 2

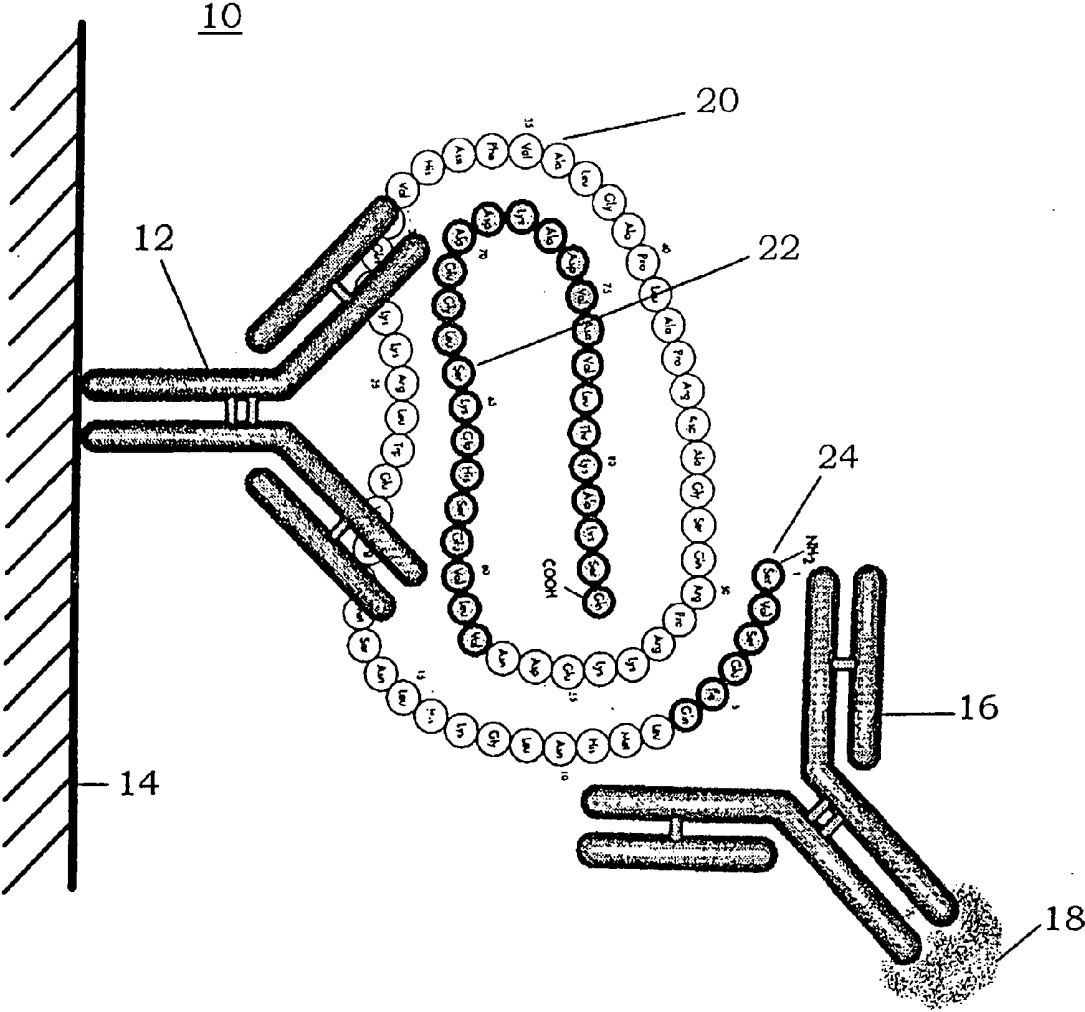
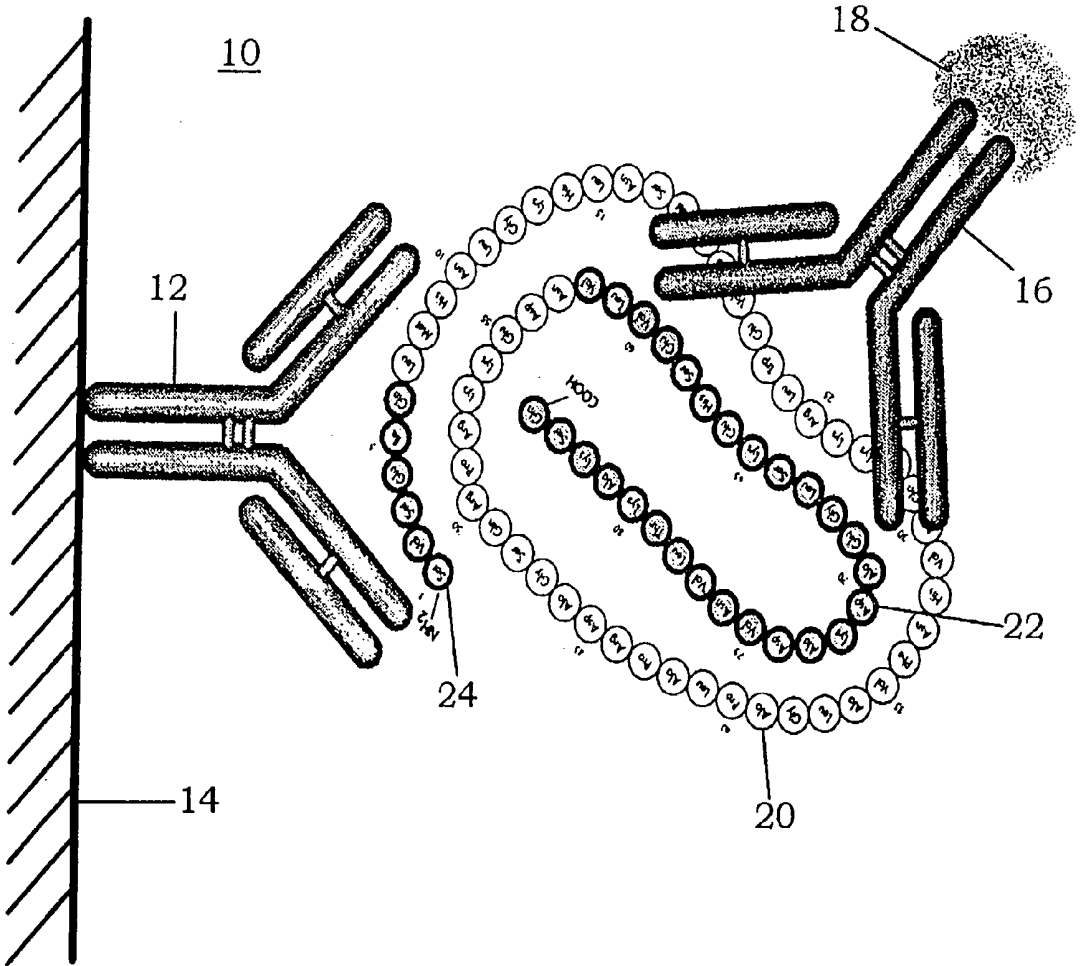
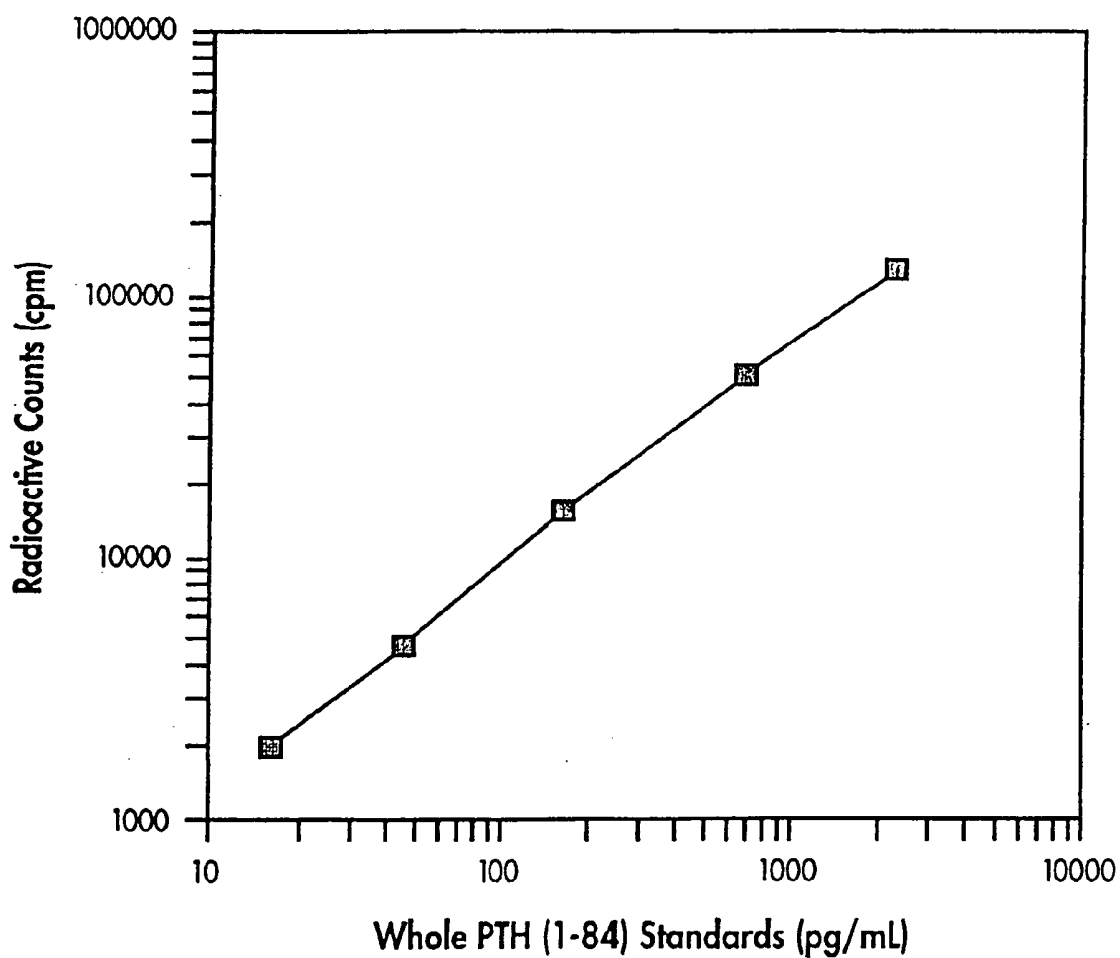


FIG. 3



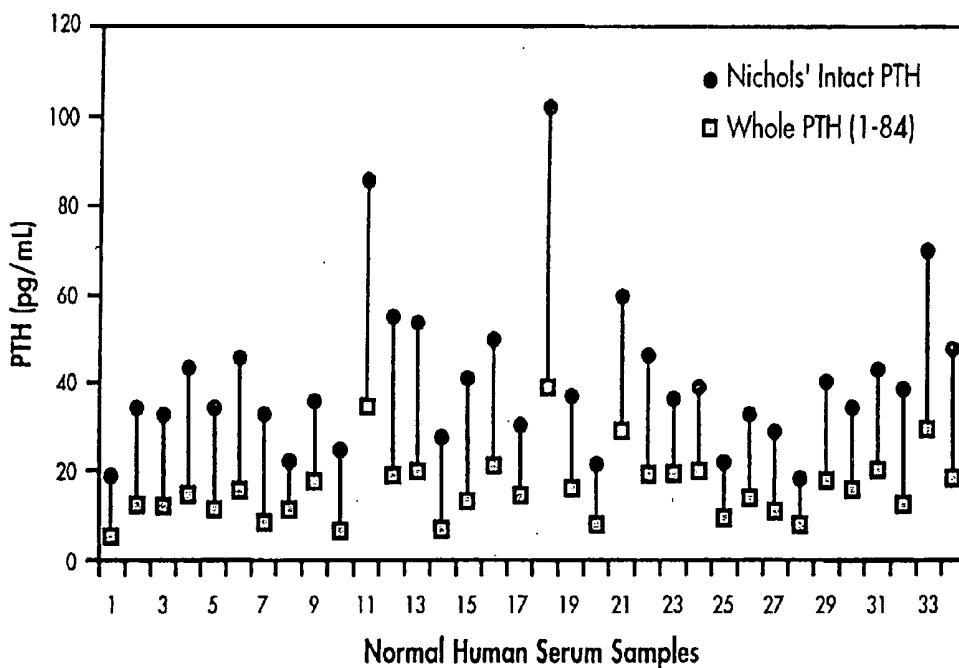
# FIG. 4

## Standard Curve for Whole PTH Assay

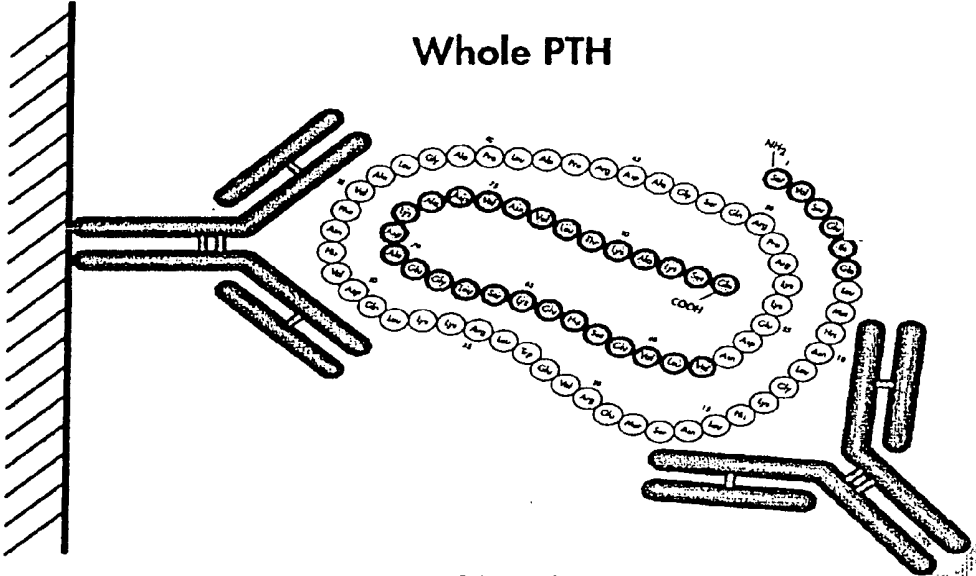


# FIG. 5

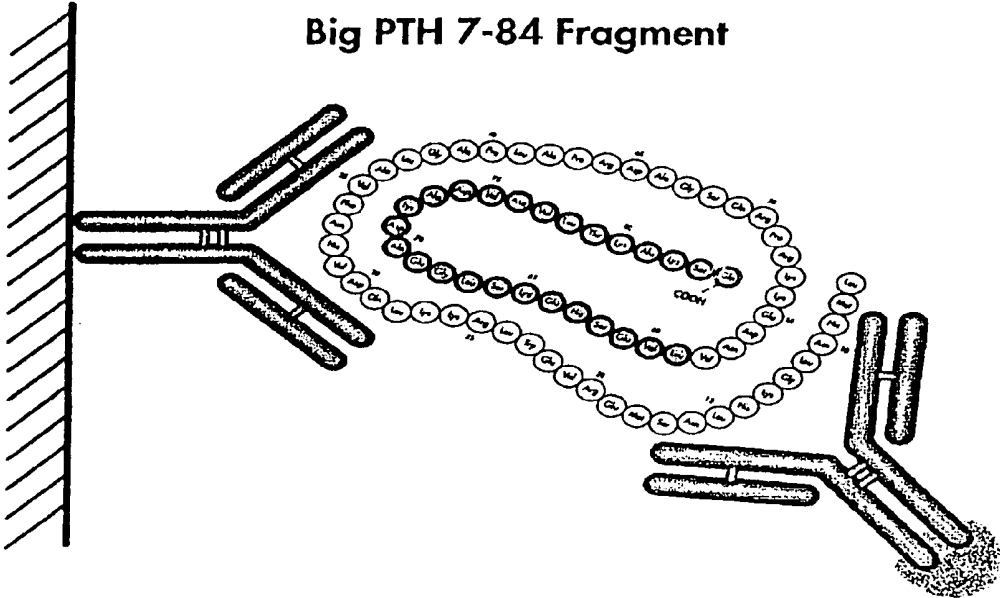
## Normal Value Comparison Whole PTH Assay (with PTH 1-8 Antibody as Tracer) versus Nichols' Intact PTH Assay (with PTH 7-84 Interference)



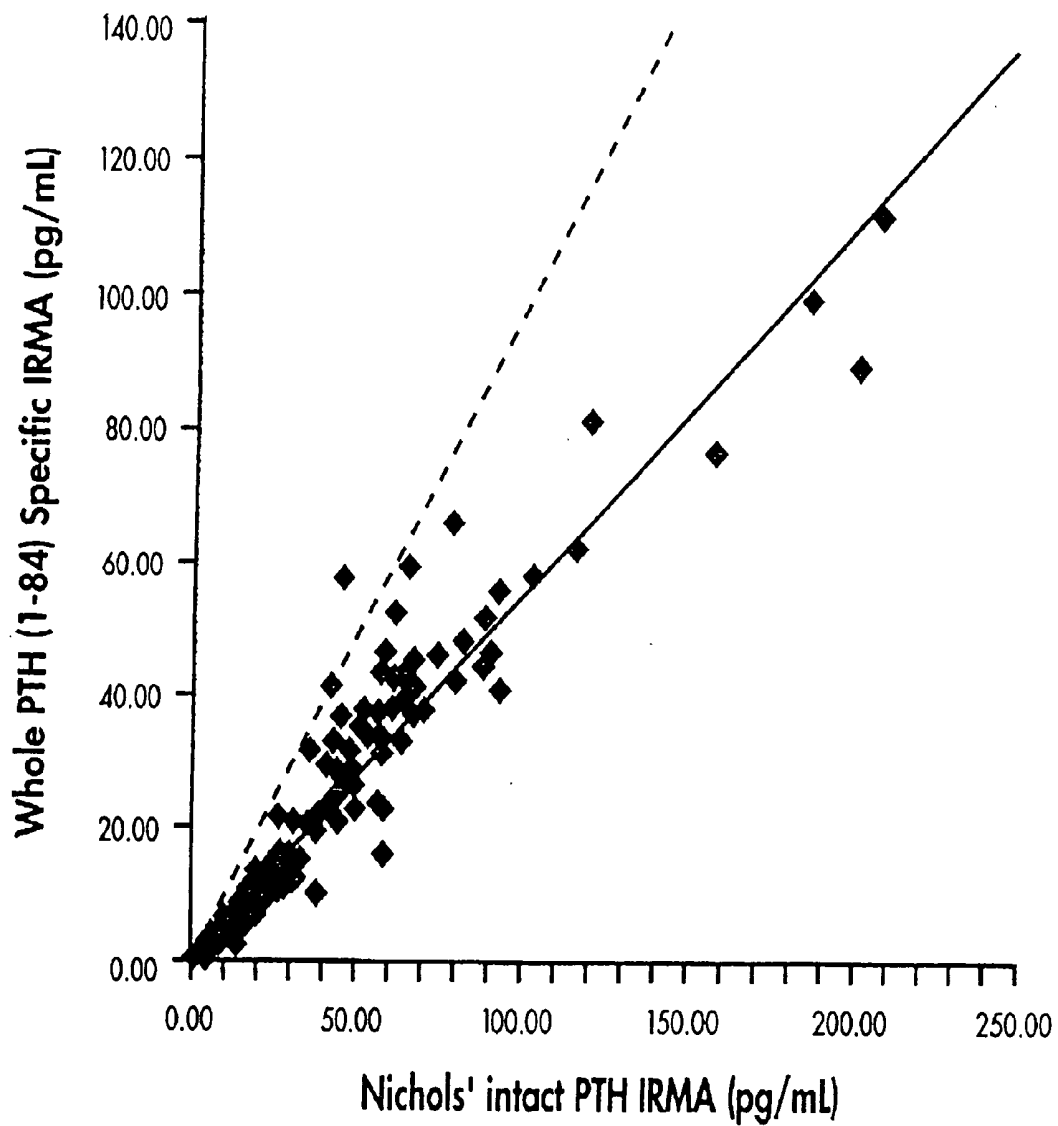
**FIG. 6A**



**FIG. 6B**



# FIG. 7



# FIG. 8

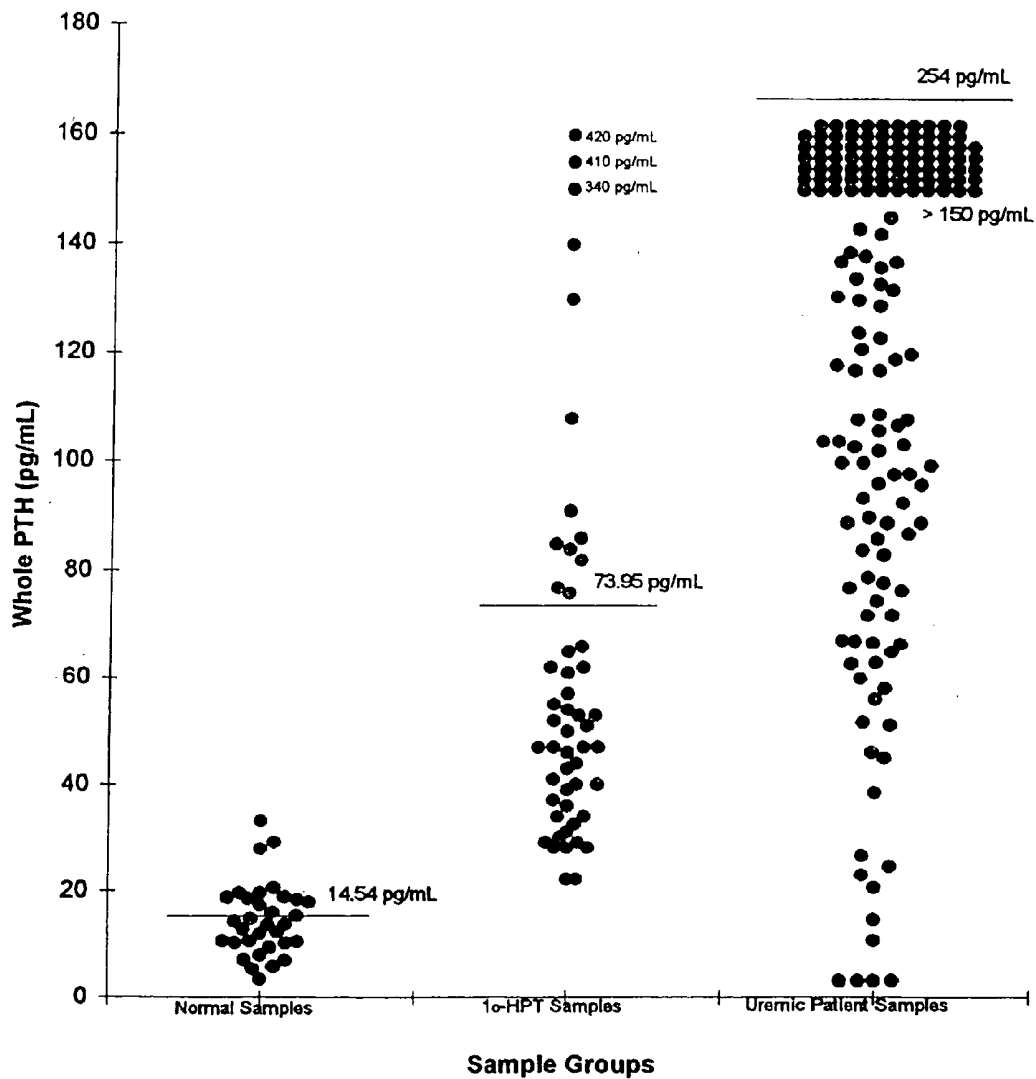
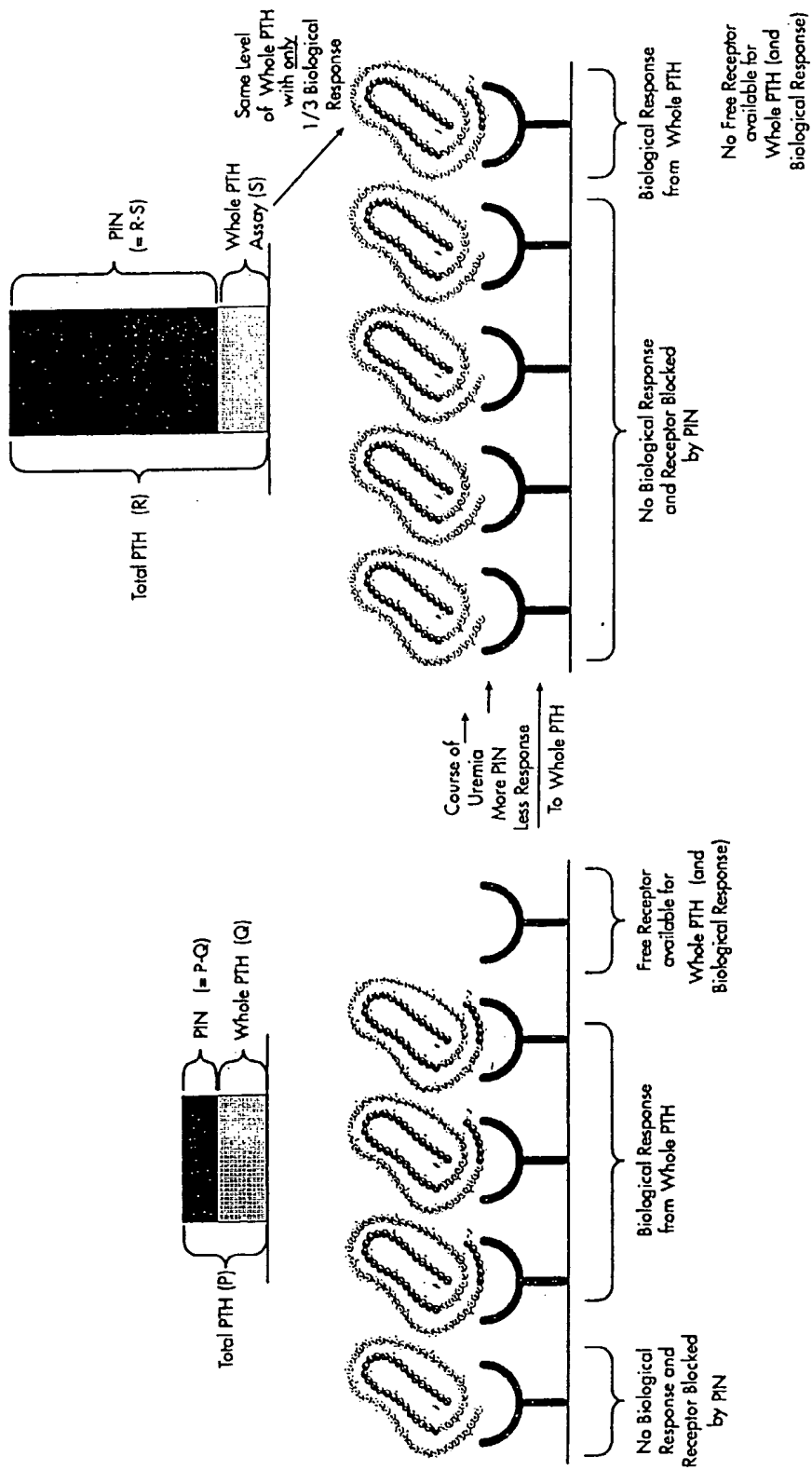
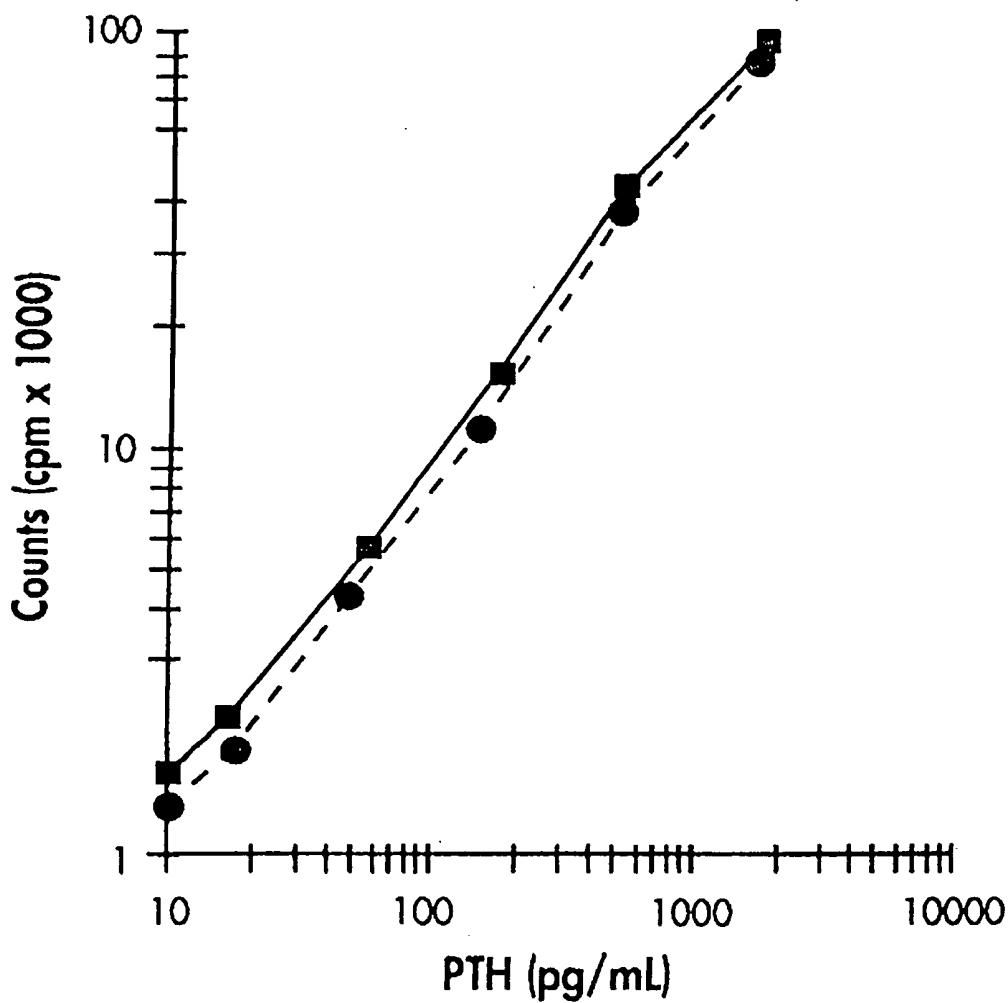


FIG. 9

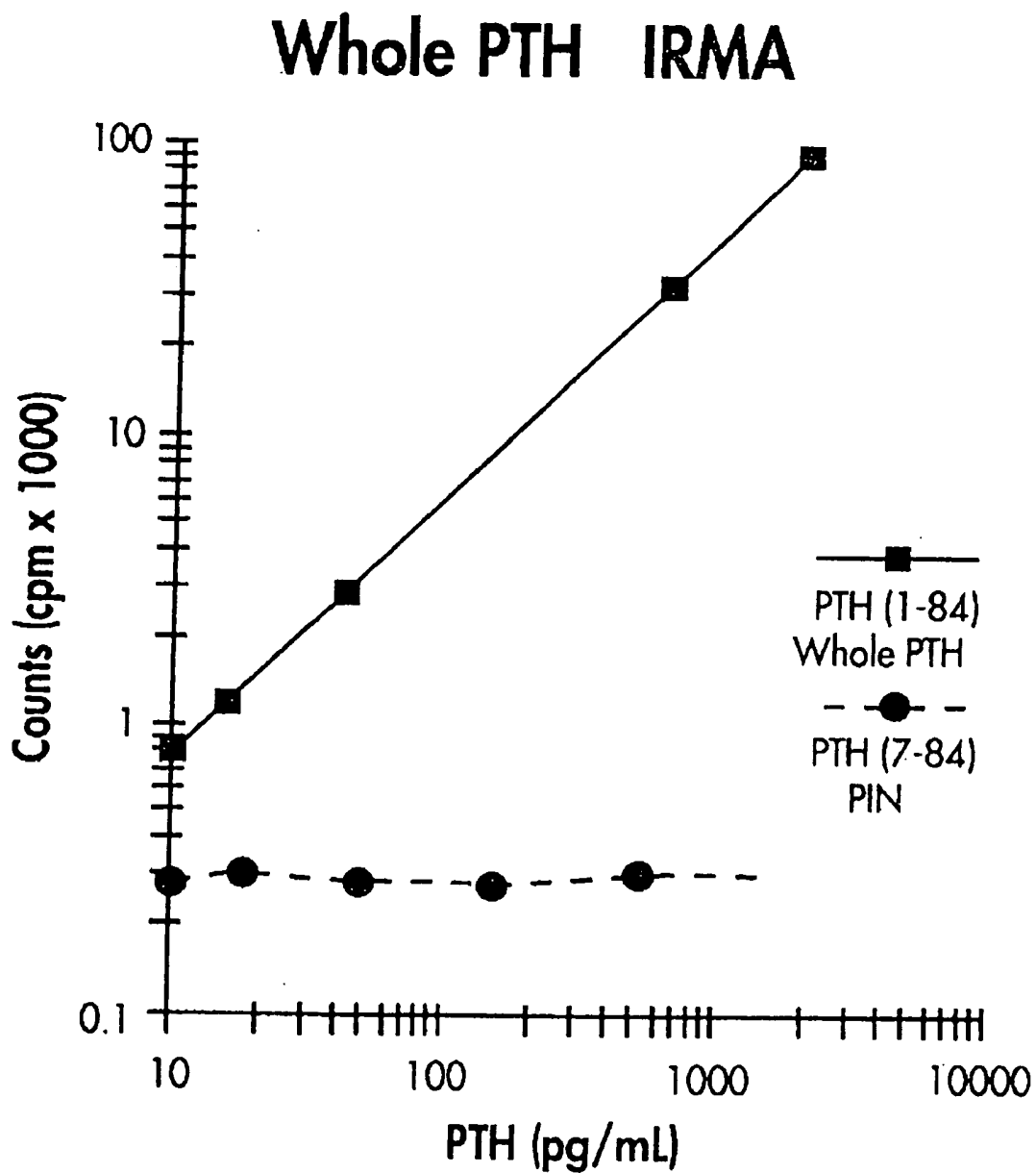


# FIG. 10

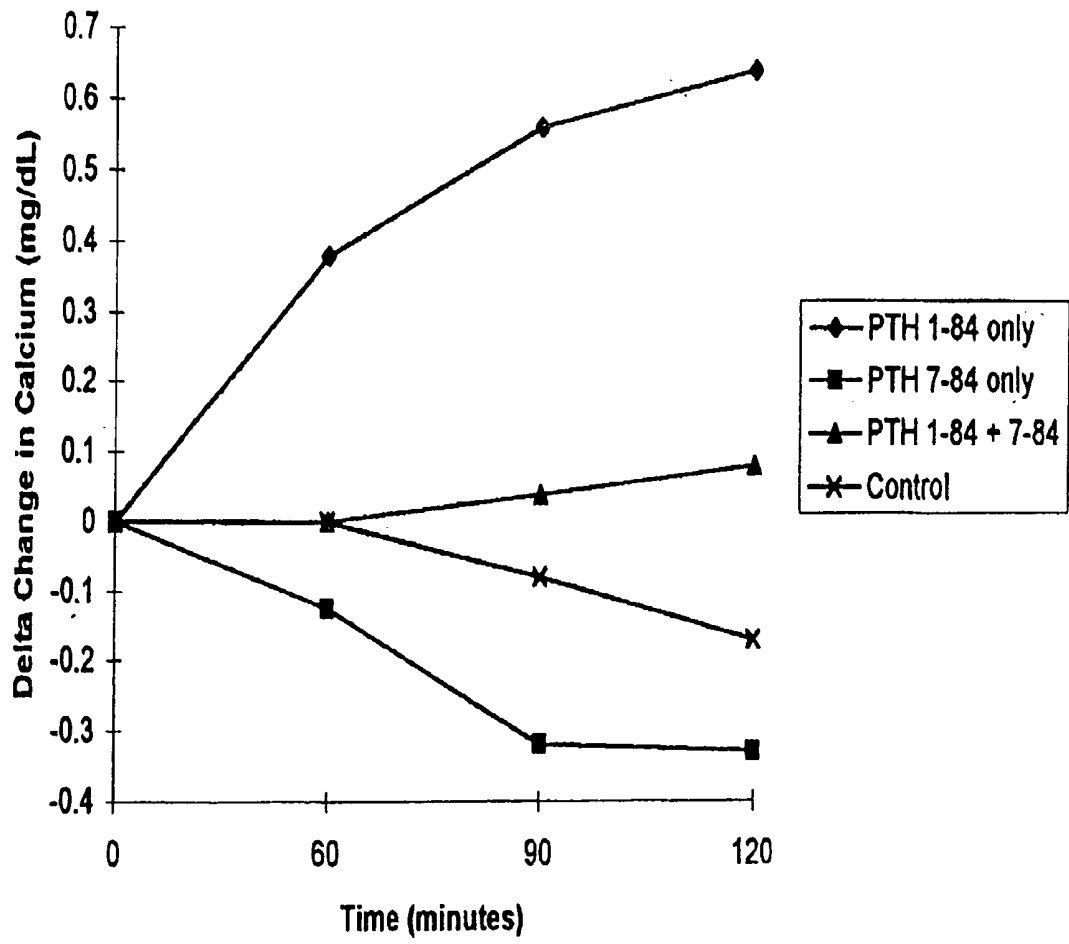
## Intact PTH IRMA - Total PTH



# FIG. 11



# FIG. 12



## METHODS FOR DIFFERENTIATING AND MONITORING PARATHYROID AND BONE STATUS RELATED DISEASES

### RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of a non-provisional utility patent application filed in the United States Patent and Trademark Office, Ser. No. 09/231, 422.

### TECHNICAL FIELD

[0002] The present invention relates to novel methods and devices for differentiating in a patient parathyroid diseases, such as hyperparathyroidism, from normal or non-disease states. One detects whole or non-fragmented (1 to 84) parathyroid hormone in a biological sample and also a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist. By either comparing values or using independently the value of either the large non-whole parathyroid hormone peptide fragment, the whole parathyroid hormone, or the combination of these values one can differentiate parathyroid and bone related disease states, as well as differentiate such states from normal states.

### BACKGROUND ART

[0003] Calcium plays an indispensable role in cell permeability, the formation of bones and teeth, blood coagulation, transmission of nerve impulse, and normal muscle contraction. The concentration of calcium ions in the blood is, along with calcitriol and calcitonin, regulated mainly by parathyroid hormone (PTH). Although calcium intake and excretion may vary, PTH serves through a feedback mechanism to maintain a steady concentration of calcium in cells and surrounding fluids. When serum calcium lowers, the parathyroid glands secrete PTH, affecting the release of stored calcium. When serum calcium increases, stored calcium release is retarded through lowered secretions of PTH.

[0004] The complete form of human PTH, sometimes referred to in the art as hPTH but referred to in the present invention either as whole PTH or wPTH, is a unique 84 amino acid peptide (SEQ ID NO. 1), as is shown in FIG. 1. Researchers have found that this peptide has an anabolic effect on bone that involves a domain for protein kinase C activation (amino acid residues 28 to 34) as well as a domain for adenylate cyclase activation (amino acid residues 1 to 7). However, various catabolic forms of clipped or fragmented PTH peptides also are found in circulation, most likely formed by intraglandular or peripheral metabolism. For example, whole PTH can be cleaved between amino acids 34 and 35 to produce a (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment. Likewise, clipping can occur between either amino acids 36 and 37 or 37 and 38. Recently, a large PTH fragment referred to as "non-(1-84) PTH" has been disclosed which is clipped closer to the N-terminal end of PTH. (See R. LePage et alia, "A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples" Clin. Chem. (1998); 44: 805-810.)

[0005] The clinical need for accurate measurement of PTH is well demonstrated. Serum PTH level is one of the most

important indices for patients with the following diseases: familial hypocalciuria; hypercalcemia; multiple endocrine neoplasia types I and II; osteoporosis; Paget's bone disease; primary hyperparathyroidism—caused by primary hyperplasia or adenoma of the parathyroid glands; pseudohypoparathyroidism; and renal failure, which can cause secondary hyperparathyroidism.

[0006] PTH plays a role in the course of disease in a patient with chronic renal failure. Renal osteodystrophy (RO) is a complex skeletal disease comprising osteitis fibrosa cystica (caused by PTH excess), osteomalacia—unmineralized bone matrix (caused by vitamin D deficiency), extraskeletal calcification/ossification (caused by abnormal calcium and phosphorus metabolism), and adynamic bone disease (contributed to by PTH suppression). Chronic renal failure patients can develop RO. Failing kidneys increase serum phosphorus (hyperphosphoremia) and decrease 1,25-dihydroxyvitamin D (1,25-D) production by the kidney. The former results in secondary hyperparathyroidism from decreased gastrointestinal calcium absorption and osteitis fibrosa cystica from increased PTH in response to an increase in serum phosphorus. The latter causes hypocalcemia and osteomalacia. With the onset of secondary hyperparathyroidism, the parathyroid gland becomes less responsive to its hormonal regulators because of decreased expression of its calcium and vitamin D receptors. Serum calcium drops. RO can lead to digital gangrene, bone pain, bone fractures, and muscle weakness.

[0007] Determining circulating biologically active PTH levels in humans has been challenging. One major problem is that PTH is found at low levels, normally 10 pg/mL to 65 pg/mL. Coupled with extremely low circulating levels is the problem of the heterogeneity of PTH and its many circulating fragments. In many cases, immunoassays have faced substantial and significant interference from circulating PTH fragments. For example, some commercially available PTH kits have almost 100% cross-reactivity with the non-(1-84) PTH fragment, (see the LePage article).

[0008] PTH immunoassays have varied over the years. One early approach is a double antibody precipitation immunoassay found in U.S. Pat. No. 4,369,138 to Arnold W. Lindall et alia. A first antibody has a high affinity for a (65-84) PTH fragment. A radioactive labeled (65-84) PTH peptide is added to the sample with the first antibody to compete for the endogenous unlabeled peptide. A second antibody is added which binds to any first antibody and radioactive labeled PTH fragment complex, thereby forming a precipitate. Both precipitate and supernatant can be measured for radioactive activity, and endogenous PTH levels can be calculated therefrom.

[0009] In an effort to overcome PTH fragment interference, immunoradiometric two-site assays for intact PTH (I-PTH) have been introduced, such as Allegro® Intact PTH assay by the Nichol's Institute of San Juan Capistrano, Calif. In one version, a capture antibody specifically binds to the C-terminal portion of hPTH while a labeled antibody specifically binds to the N-terminal portion of the captured hPTH. In another, two monoclonal antibodies were used, both of which attached to the N-terminal portion of hPTH. Unfortunately, these assays have problems in that they measure but do not discriminate between wPTH and non-whole PTH peptide fragments. This inability comes to the

fore in hyperparathyroid patients and renal failure patients who have significant endogenous concentrations of large, non-whole PTH fragments.

**[0010]** Recently, researchers have made a specific binding assay directed to the large N-terminal PTH fragments. (See Gao, Ping et alia "*Immunochemiluminometric assay with two monoclonal antibodies against the N-terminal sequence of human parathyroid hormone*", Clinica Chimica Acta 245 (1996) 39-59.) This immunochemiluminometric assay uses two monoclonal antibodies to detect N-terminal (1-34) PTH fragments but not mid-portion PTH fragments or C-terminal PTH fragments. A key factor in the design of these assays is to eliminate any reaction with C-terminal PTH fragments.

#### DISCLOSURE OF THE INVENTION

**[0011]** The present invention relates to novel methods and devices for differentiating in a patient parathyroid diseases, (such as primary hyperparathyroidism, secondary hyperparathyroidism, and stages thereof), from normal or non-disease states; for monitoring the function of parathyroid glands either during or after treatment, i.e., intra-operation and after operation parathyroid function monitoring as well as therapeutic treatment; and also for monitoring the effects of therapeutic treatments for parathyroid related bone diseases and hyperparathyroidism. One detects the level in the serum or blood of at least one of three different parameters, namely, whole or non-fragmented parathyroid hormone in a biological sample, a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist, or the combination of the two values. By comparing the two values or by examining independently one of the above three values, one can differentiate parathyroid and bone disease states, as well as differentiate such states from normal states, as the relationship between these values, as well as the values themselves, change significantly between a normal person and a patient with a parathyroid disease.

**[0012]** The present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No. 2 [PTH<sub>3-84</sub>]) and (SEQ ID No. 3 [PTH<sub>34-84</sub>]), functions in vivo as a wPTH antagonist or inhibitor (PIN), (see FIG. 12). In other words, the binding of wPTH to PTH receptors and the subsequent biological activity are affected by the presence of this PIN peptide fragment. The PTH receptors can be tied up with respect to PTH or PTH analogs in that the PTH binding site is blocked. The relationship between the concentrations of wPTH and PIN vary with PTH related disease states, and thus, are indicative of such states. Equally useful in view of the discovery of the antagonist nature of PIN, the present invention relates to novel methods and devices for monitoring parathyroid related bone diseases, and resultant bone loss or build-up. Increased amounts of PIN can inhibit the calcium releasing activity of PTH.

**[0013]** In making a measurement of wPTH, one does not want to detect PIN. The method for measuring the amount of wPTH in a sample such as serum, plasma, or blood comprises four general steps which can vary depending upon whether one uses a first antibody or antibody fragment specific for the PTH peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID No. 4), wherein at least four amino acids are part of the antibody reactive portion of the peptide either as a signal antibody or a capture antibody in

conventional immunoassay formats. (One can also use an analogous peptide present in other species, such as a rat peptide in which the first amino acid serine is substituted with an alanine.) Used either as a signal antibody or as a capture antibody, enough antibody is added to bind all wPTH present. Next, one allows the first antibody to bind to any wPTH present, thereby forming a complex. A specific binding label comprised of a second antibody and a conventional immunoassay label, such as chemiluminescent agents, calorimetric agents, energy transfer agents, enzymes, fluorescent agents, and radioisotopes, is used to label the complex, preferably at the C-terminal end of wPTH, and can be added either substantially simultaneously with the first antibody or subsequent thereto. Finally, one uses conventional techniques to measure the amount of labeled complex, and thereby calculate wPTH levels in the sample. If used as a signal antibody, then the first antibody still attaches at the N-terminal end, but the second antibody would serve as a capture antibody that attaches at the C-terminal end.

**[0014]** In making a measurement of PIN, one can either measure it directly, or indirectly. An indirect measurement can be made by first measuring wPTH and then measuring total PTH. Subtracting the wPTH value from the total PTH value, one derives the PIN value. (For the purposes of the present invention, "total PTH" refers to the sum of wPTH, the naturally occurring predominant PTH receptor binding agonist, and PIN, the naturally occurring predominant PTH receptor binding antagonist.) A total PTH assay detects both PIN and wPTH by detecting the N-terminal end of PTH not at SEQ ID No. 4, the very end of the N-terminal. By detecting between about amino acids 7 to 38 of PTH, the assay can detect both. A commercially available assay for total PTH is available from Scantibodies Laboratory, Inc. of Santee, Calif. A direct measurement of total PTH can be made by using an antibody or antibody fragment specific for a portion of the PTH peptide LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ALA-SER-VAL-GLU-ARG-MET-GLN-TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL ALA-LEU-GLY (SEQ ID No. 5), which comprises amino acids 7 to 38 of PTH, (preferably between amino acids 9 to 34), wherein at least four amino acids are part of the antibody reactive portion of the peptide. Such an antibody or antibody fragment can be used in conventional immunoassay formats either as a signal antibody or a capture antibody.

**[0015]** To differentiate between parathyroid disease states and the normal state or to monitor the effects of therapeutic treatment for parathyroid disease states, one can compare the relationship between the values of wPTH, PIN, or total PTH, (the combination of wPTH and PIN), in other words, the relationship between the values of PIN and total PTH, between PIN and whole PTH, or between whole PTH and total PTH. For example, one can use a proportion between wPTH and total PTH, between PIN and total PTH, or between PIN and wPTH. (Comparisons can even take the form of a neural network of all these factors.) Regardless of the comparative method chosen, these values change significantly between a normal person and a patient with a parathyroid disease and between various stages of parathyroid diseases.

**[0016]** Alternatively, one can either differentiate between parathyroid disease states and the normal state or monitor the effects of therapeutic treatment for parathyroid disease

states by examining independently the value of either wPTH, PIN, or total PTH alone.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 is a diagrammatic view of human wPTH.

[0018] FIG. 2 is a diagrammatic view of a wPTH assay using the present antibody as a tracer element.

[0019] FIG. 3 is a diagrammatic view of a wPTH assay using the present antibody as a capture element.

[0020] FIG. 4 is a graph showing a standard curve for a wPTH assay.

[0021] FIG. 5 is a graph comparing a conventional I-PTH assay with the present wPTH assay for healthy normal persons with "normal" PTH values.

[0022] FIG. 6 is a diagrammatic view showing interference from PIN in conventional I-PTH assays.

[0023] FIG. 7 is a graph comparing a conventional I-PTH assay with the present wPTH assay for patients with chronic uremia.

[0024] FIG. 8 is a graph showing the distribution of wPTH values for healthy normal persons, patients with primary hyperparathyroidism, and patients with chronic uremia.

[0025] FIG. 9 is a diagrammatic view showing how PIN blocks the action of wPTH at the receptor level, thereby making the person insensitive to the biological effects of wPTH.

[0026] FIG. 10 is a graph demonstrating complete cross-reactivity of wPTH and PIN in a total PTH assay used in the present invention.

[0027] FIG. 11 is a graph demonstrating how the whole PTH assay used in the present invention does not detect to PIN. FIG. 12 is a graph demonstrating how PIN is an in vivo inhibitor of wPTH.

#### BEST MODES FOR CARRYING OUT THE INVENTION

[0028] In disclosing the present invention, one should remember that there are a number of closely analogous, species dependent forms of PTH. The amino acid sequence of hPTH is shown in FIG. 1. However, for rat PTH, bovine PTH, or porcine PTH, for example, one finds the substitutions at some of the amino acids in the hPTH sequence. For the purposes of the present invention, one can use interchangeably antibodies or antibody fragments to forms of these PTHs, although it is preferred to use an antibody with specificity for PTH having a sequence matching the species in which the PTH measurements are made.

#### Whole PTH Immunoassay

[0029] A preferred embodiment of the present invention is an immunoradiometric assay 15 (IRMA), often referred to as a sandwich assay, as shown FIGS. 2 and 3. Elements employed in such an assay (10) include a capture antibody (12) attached to a solid support (14) and a signal antibody (16) having a label (18), attached thereto (20). Typically, one selects a capture antibody that is specific for C-terminal PTH fragments (22), while the label antibody is specific for the

initial wPTH peptide sequence which comprises a domain for adenylate cyclase activation (24), as shown in FIG. 2. However, one could reverse the specificity of these antibodies, as is shown in FIG. 3.

[0030] Alternatively, one could create an immunoassay in which wPTH is either precipitated from solution or otherwise differentiated in a solution, as in conventional precipitating assays or turbidometric assays. For example, one can use at least three antibodies to form a precipitating mass. In addition to the initial wPTH sequence antibody and a C-terminal antibody, one can use at least a third antibody which attaches to the mid portion of PTH. The combined mass of wPTH and the at least three antibodies would form a labeled precipitating mass which can be measured by conventional techniques. Another method would be to couple the initial wPTH sequence antibody to colloidal solid supports, such as latex particles.

[0031] More specifically, one can create a signal antibody by iodinating 50 micrograms of affinity purified goat anti-(1-6) PTH antibody (Scantibodies Laboratory, Inc., Santee California, U.S.A.) by oxidation with chloramine T, incubation for 25 seconds at room temperature with 1 millicurie of 125-1 radioisotope and reduction with sodium metabisulfate. Unincorporated 125-1 radioisotope is separated from the 125-1-Goat anti-(1-6) PTH signal antibody by, passing the iodination mixture over a PD-10 desalting column (Pharmacia, Uppsala, Sweden) and following the manufacturers instructions. The fractions collected from the desalting column are measured in a gamma counter and those fractions representing the 125-1-goat anti-(1-6) PTH antibody are pooled and diluted to approximately 300,000 D PM (disintegrations per minute) per 100 microliters. This solution is the tracer solution to be used in the whole PTH IRMA.

[0032] Capture antibody coated tubes can be created by attaching affinity purified goat anti PTH 39-84 antibody, (Scantibodies Laboratory, Inc., Santee, Calif., U.S.A.), to 12x75 mm polystyrene tubes (Nunc, Denmark) by means of passive absorption techniques which are known to those of skill in the art. The tubes are emptied and dried, creating solid phase antibody coated tubes.

[0033] To conduct a whole PTH assay of a sample, 200 microliter samples of human serum are added to the solid phase antibody coated tubes. To each tube is added 100 microliters of the tracer solution (labeled goat anti-(1-6) PTH signal antibody). The tubes are incubated at room temperature with shaking at 170 rpm for 20-22 hours. During this time the immunochemical reaction of forming the sandwich of {solid phase goat anti-(39-84) PTH antibody}—{whole PTH}—{125-1-goat anti-(1-6) PTH antibody} takes place. Following this incubation, the test tubes are washed with distilled water. Radioactivity on the solid phase, which amount corresponds to the quantity of wPTH present, is measured using a gamma counter. The radioactivity data for the samples is processed by conventional analysis with use of the results from standards and controls and computer software in order that the concentration of whole PTH in the samples may be ascertained. FIG. 4 shows a standard curve for such an assay.

#### Initial Whole PTH Sequence Peptide

[0034] In order to make the signal antibody in the above assay, first one makes a synthetic PTH peptide correspond-

ing either to hPTH (Ser-Val-Ser-Glu-Ile-Gln-Leu-Met), rat PTH (Ala-Val-Ser-Glu-Ile-Gln-Leu-Met), or at least four amino acids in the common sequence. The selected peptide can play two roles in making an assay, first as a specific source for creating a polyclonal antibody or monoclonal antibody source for signal antibody or capture antibody, and second as part of an affinity purification means for isolating the desired signal antibody or capture antibody.

**[0035]** Briefly, such a peptide can be synthesized on an Applied Biosystems, Inc. (Foster City, Calif., U.S.A.) Model 431 automated peptide synthesizer employing Fmoc (9-fluoronylmethoxycarbonyl) as the alpha-amino protecting group. All amino acids and solvents are from Applied Biosystems and are of synthesis grade. Following synthesis, the peptide is cleaved from the resin, and side chains are de-protected, using a cleavage cocktail containing 6.67% phenol, 4.4% (v/v) thioanisole and 8.8% ethanedithiol in trifluoroacetic acid (TFA). The cleaved peptide is precipitated and washed several times in cold diethyl ether. It is then dissolved in water and lyophilized. The crude peptide is subjected to amino acid analysis (Waters PICO-TAG System, Boston, Mass., U.S.A.) and reversed-phase HPLC using a VYDAC (™) C8 column with 0.1% TFA in water and 99.9% acetonitrile in 0.1% TFA as the mobile buffers. The presence of a single major peak along with the appropriate amino acid composition is taken as evidence that the peptide is suitable for further use.

**[0036]** The resulting peptide is then attached to cross linked agarose beads (activated Sepharose 4B from Pharmacia, Uppsala, Sweden) according to instructions from the manufacturer. Armed with the initial peptide sequence on a bead, one can affinity purify a polyclonal antibody serum source to isolate the initial sequence antibody for the wPTH immunoassay.

#### Initial Sequence Whole PTH Antibody

**[0037]** To create an affinity-purified anti-(1-6) PTH antibody, one first uses a selected initial PTH sequence peptide as described above as part of an immunogen for injection into a goat. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000, or as part of the wPTH complete sequence. The immunogen is mixed with an equal volume of Freund's complete adjuvant which is a mixture of light mineral oil, Arlacial detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which is rich in (1-6) PTH antibodies.

**[0038]** To purify the antiserum for the desired (1-6) PTH antibody, one packs a separation column with the initial PTH sequence peptide bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the (1-6) PTH specificity. The bound specific goat

anti-(1-6) PTH polyclonal antibody is eluted from the solid phase PTH 1-6 in the column by passing an elution solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0.01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

#### Comparison Between Whole PTH and Total PTH Assays

**[0039]** The present wPTH IRMA assay was compared to a conventional intact PTH or I-PTH immunoassay, the Allegro Nichols Intact-PTH assay, (which is commercially available and made by Nichols Institute Diagnostics of San Juan Capistrano, Calif., U.S.A.), in both PTH normal persons and those suffering from chronic uremia. This I-PTH immunoassay, due to its 100% cross reactivity between PIN and wPTH, is in actuality a total PTH assay, (see **FIG. 10**).

**[0040]** **FIG. 5** shows the results for 34 normal human serum samples from healthy subjects which were assayed both by the present wPTH IRMA and the above I-PTH assay. In every case, the level of wpm detected by the IRMA is lower than that reported by the I-PTH assay, demonstrating the ability of the present IRMA to avoid detecting the interfering large, non (1-84) PTH fragment detected by the I-PTH assay, (see **FIG. 11**). **FIG. 6** illustrates how such interference can occur. An N-terminal PTH specific signal antibody which is not specific to the initial PTH peptide sequence, as in the present invention, can detect not only wPTH (as in the upper part of **FIG. 6**), but also can detect PIN, the large, non (1-84) PTH fragment, (as in the lower part of **FIG. 6**).

**[0041]** A comparison of assay results for 157 chronic uremic patients is shown in **FIG. 7**. Serum samples from these patients were measured using the wPTH IRMA and the above I-PTH assay. In every case the wPTH levels are lower than I-PTH values.

#### Clinical Use

**[0042]** The present wPTH and PIN assays have been used in a clinical setting involving 188 persons. The group included 31 persons having normal healthy parathyroid glands and 57 patients with chronic uremia who are undergoing dialysis on a continuous basis. Each person had a blood sample drawn which was assayed using a wPTH assay from Scantibodies Laboratory, Inc. as well as an I-PTH assay from Nichols Institute which gave total PTH values.

**[0043]** Table I shows the results individually and comparatively, of the wPTH, PIN, and total PTH assays from chronic uremic patients on dialysis.

TABLE 1

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 1           | 1410            | 740             | 670       | 48%              | 91%              | 52%                    |
| 2           | 185             | 89              | 96        | 52%              | 108%             | 48%                    |
| 3           | 231             | 104             | 127       | 55%              | 122%             | 45%                    |
| 4           | 1020            | 590             | 430       | 42%              | 73%              | 53%                    |
| 5           | 270             | 159             | 111       | 41%              | 70%              | 59%                    |
| 6           | 201             | 100             | 101       | 50%              | 101%             | 50%                    |
| 7           | 380             | 100             | 280       | 74%              | 280%             | 26%                    |
| 8           | 460             | 277             | 183       | 40%              | 66%              | 60%                    |

TABLE 1-continued

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 9           | 380             | 197             | 183       | 48%              | 93%              | 52%                    |
| 10          | 880             | 522             | 358       | 41%              | 69%              | 59%                    |
| 11          | 310             | 154             | 156       | 50%              | 101%             | 50%                    |
| 12          | 880             | 451             | 429       | 49%              | 95%              | 51%                    |
| 13          | 670             | 418             | 252       | 38%              | 60%              | 63%                    |
| 14          | 390             | 221             | 169       | 43%              | 76%              | 57%                    |
| 15          | 170             | 108             | 62        | 36%              | 57%              | 64%                    |
| 16          | 510             | 381             | 129       | 25%              | 34%              | 75%                    |
| 17          | 200             | 67              | 133       | 67%              | 199%             | 34%                    |
| 18          | 170             | 109             | 61        | 36%              | 56%              | 64%                    |
| 19          | 360             | 199             | 161       | 45%              | 81%              | 55%                    |
| 20          | 260             | 164             | 96        | 37%              | 59%              | 63%                    |
| 21          | 440             | 372             | 68        | 15%              | 18%              | 85%                    |
| 22          | 120             | 51.7            | 68.3      | 57%              | 132%             | 43%                    |
| 23          | 600             | 527             | 73        | 12%              | 14%              | 83%                    |
| 24          | 220             | 130             | 90        | 41%              | 69%              | 59%                    |
| 25          | 190             | 136             | 54        | 28%              | 40%              | 72%                    |
| 26          | 220             | 118             | 102       | 46%              | 86%              | 54%                    |
| 27          | 630             | 334             | 296       | 47%              | 89%              | 53%                    |
| 28          | 150             | 90              | 60        | 40%              | 67%              | 60%                    |
| 29          | 170             | 106             | 64        | 38%              | 60%              | 62%                    |
| 30          | 810             | 489             | 321       | 40%              | 66%              | 60%                    |
| 31          | 570             | 319             | 251       | 44%              | 79%              | 56%                    |
| 32          | 570             | 467             | 103       | 18%              | 22%              | 82%                    |
| 33          | 400             | 300             | 100       | 25%              | 33%              | 75%                    |
| 34          | 560             | 378             | 182       | 33%              | 48%              | 68%                    |
| 35          | 310             | 121             | 189       | 61%              | 156%             | 39%                    |
| 36          | 240             | 98              | 142       | 59%              | 145%             | 41%                    |
| 37          | 280             | 133             | 157       | 54%              | 118%             | 48%                    |
| 38          | 230             | 124             | 106       | 46%              | 85%              | 54%                    |
| 39          | 350             | 319             | 31        | 9%               | 10%              | 91%                    |
| 40          | 200             | 133             | 67        | 34%              | 50%              | 67%                    |
| 41          | 920             | 564             | 356       | 39%              | 63%              | 61%                    |
| 42          | 210             | 89              | 121       | 58%              | 136%             | 42%                    |
| 43          | 1990            | 904             | 1086      | 55%              | 120%             | 45%                    |
| 44          | 300             | 212             | 88        | 29%              | 42%              | 71%                    |
| 45          | 260             | 132             | 128       | 49%              | 97%              | 51%                    |
| 46          | 140             | 72              | 68        | 49%              | 94%              | 51%                    |
| 47          | 250             | 129             | 121       | 48%              | 94%              | 52%                    |
| 48          | 130             | 72              | 58        | 45%              | 81%              | 56%                    |
| 49          | 1840            | 1000            | 840       | 46%              | 84%              | 54%                    |
| 50          | 280             | 167             | 113       | 40%              | 68%              | 60%                    |
| 51          | 490             | 268             | 222       | 45%              | 83%              | 55%                    |
| 52          | 150             | 77.1            | 72.9      | 49%              | 95%              | 51%                    |
| 53          | 140             | 58.1            | 81.9      | 59%              | 141%             | 42%                    |
| 54          | 210             | 92.7            | 117.3     | 56%              | 127%             | 44%                    |
| 55          | 160             | 79              | 81        | 51%              | 103%             | 49%                    |
| 56          | 480             | 296             | 184       | 38%              | 62%              | 62%                    |
| 57          | 480             | 281             | 199       | 41%              | 71%              | 59%                    |
| 58          | 270             | 120             | 150       | 56%              | 125%             | 44%                    |
| 59          | 97              | 45              | 52        | 54%              | 116%             | 46%                    |
| 60          | 330             | 154             | 176       | 53%              | 114%             | 47%                    |
| 61          | 110             | 56              | 54        | 49%              | 96%              | 51%                    |
| 62          | 660             | 456             | 204       | 31%              | 45%              | 69%                    |
| 63          | 300             | 137             | 163       | 54%              | 119%             | 46%                    |
| 64          | 240             | 145             | 95        | 40%              | 66%              | 60%                    |
| 65          | 100             | 66.5            | 33.5      | 34%              | 50%              | 67%                    |
| 66          | 410             | 416.3           | -6.3      | -2%              | -2%              | 102%                   |
| 67          | 410             | 235.7           | 174.3     | 43%              | 74%              | 57%                    |
| 68          | 45              | 14.4            | 30.6      | 68%              | 213%             | 32%                    |
| 69          | 200             | 102.3           | 97.7      | 49%              | 96%              | 51%                    |
| 70          | 300             | 134             | 166       | 55%              | 124%             | 45%                    |
| 71          | 320             | 202             | 118       | 37%              | 58%              | 63%                    |
| 72          | 440             | 254             | 186       | 42%              | 73%              | 58%                    |
| 73          | 190             | 99.6            | 90.4      | 48%              | 91%              | 52%                    |
| 74          | 160             | 74.6            | 85.4      | 53%              | 114%             | 47%                    |
| 75          | 600             | 429.8           | 170.2     | 28%              | 40%              | 72%                    |
| 76          | 1140            | 632             | 508       | 45%              | 80%              | 55%                    |
| 77          | 440             | 211             | 229       | 52%              | 109%             | 48%                    |
| 78          | 450             | 276             | 174       | 39%              | 63%              | 61%                    |
| 79          | 510             | 344             | 166       | 33%              | 48%              | 67%                    |
| 80          | 190             | 62.8            | 127.2     | 67%              | 203%             | 33%                    |

TABLE 1-continued

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 81          | 170             | 86              | 84        | 49%              | 98%              | 51%                    |
| 82          | 180             | 103.4           | 76.6      | 43%              | 74%              | 57%                    |
| 83          | 78              | 22.7            | 55.3      | 71%              | 244%             | 29%                    |
| 84          | 230             | 117             | 113       | 49%              | 97%              | 51%                    |
| 85          | 160             | 96              | 64        | 40%              | 67%              | 60%                    |
| 86          | 220             | 89              | 131       | 60%              | 147%             | 40%                    |
| 87          | 470             | 321.5           | 148.5     | 32%              | 46%              | 68%                    |
| 88          | 310             | 137             | 173       | 56%              | 126%             | 44%                    |
| 89          | 2050            | 1127            | 923       | 45%              | 82%              | 55%                    |
| 90          | 930             | 414             | 516       | 55%              | 125%             | 45%                    |
| 91          | 180             | 65              | 115       | 64%              | 177%             | 36%                    |
| 92          | 560             | 238             | 322       | 58%              | 135%             | 43%                    |
| 93          | 640             | 597             | 43        | 7%               | 7%               | 93%                    |
| 94          | 590             | 382             | 208       | 35%              | 54%              | 65%                    |
| 95          | 270             | 103             | 167       | 62%              | 162%             | 38%                    |
| 96          | 560             | 349             | 211       | 38%              | 60%              | 62%                    |
| 97          | 180             | 78              | 102       | 57%              | 131%             | 43%                    |
| 98          | 790             | 429             | 361       | 46%              | 84%              | 54%                    |
| 99          | 670             | 372             | 298       | 44%              | 80%              | 56%                    |
| 100         | 140             | 20.4            | 119.6     | 85%              | 586%             | 15%                    |
| 101         | 190             | 117             | 73        | 38%              | 62%              | 62%                    |
| 102         | 190             | 108             | 82        | 43%              | 76%              | 57%                    |
| 103         | 430             | 217             | 213       | 50%              | 98%              | 50%                    |
| 104         | 560             | 439             | 121       | 22%              | 28%              | 78%                    |
| 105         | 500             | 357.7           | 142.3     | 28%              | 40%              | 72%                    |
| 106         | 1560            | 777             | 783       | 50%              | 101%             | 50%                    |
| 107         | 62              | 24.3            | 37.7      | 61%              | 155%             | 39%                    |
| 108         | 430             | 226             | 204       | 47%              | 90%              | 53%                    |
| 109         | 160             | 67.2            | 92.8      | 58%              | 138%             | 42%                    |
| 110         | 530             | 346             | 184       | 35%              | 53%              | 65%                    |
| 111         | 260             | 142             | 118       | 45%              | 83%              | 55%                    |
| 112         | 580             | 163             | 417       | 72%              | 256%             | 28%                    |
| 113         | 440             | 579             | -139      | -32%             | -24%             | 132%                   |
| 114         | 500             | 232.3           | 267.7     | 54%              | 115%             | 46%                    |
| 115         | 160             | 60              | 100       | 63%              | 167%             | 38%                    |
| 116         | 340             | 202             | 138       | 41%              | 68%              | 59%                    |
| 117         | 260             | 138             | 122       | 47%              | 88%              | 53%                    |
| 118         | 260             | 119             | 141       | 54%              | 118%             | 46%                    |
| 119         | 160             | 84              | 76        | 48%              | 90%              | 53%                    |
| 120         | 130             | 46              | 84        | 65%              | 183%             | 35%                    |
| 121         | 190             | 104             | 86        | 45%              | 83%              | 55%                    |
| 122         | 420             | 334             | 86        | 20%              | 26%              | 80%                    |
| 123         | 630             | 440             | 190       | 30%              | 43%              | 70%                    |
| 124         | 75              | 26.4            | 48.6      | 65%              | 184%             | 35%                    |
| 125         | 260             | 143             | 117       | 45%              | 82%              | 55%                    |
| 126         | 640             | 409             | 231       | 36%              | 56%              | 64%                    |
| 127         | 130             | 66.7            | 63.3      | 49%              | 95%              | 51%                    |
| 128         | 700             | 381             | 319       | 46%              | 84%              | 54%                    |
| 129         | 560             | 376             | 184       | 33%              | 49%              | 67%                    |
| 130         | 240             | 107             | 133       | 55%              | 124%             | 45%                    |
| 131         | 110             | 63              | 47        | 43%              | 75%              | 57%                    |
| 132         | 420             | 297             | 123       | 29%              | 41%              | 71%                    |
| 133         | 580             | 229             | 351       | 61%              | 153%             | 39%                    |
| 134         | 310             | 201.2           | 108.8     | 35%              | 54%              | 65%                    |
| 135         | 160             | 97.9            | 62.1      | 39%              | 63%              | 61%                    |
| 136         | 290             | 138.7           | 151.3     | 52%              | 109%             | 48%                    |
| 137         | 200             | 96.2            | 103.8     | 52%              | 108%             | 48%                    |
| 138         | 770             | 662.7           | 107.3     | 14%              | 16%              | 86%                    |
| 139         | 290             | 130.7           | 159.3     | 55%              | 122%             | 45%                    |
| 140         | 260             | 219             | 41        | 16%              | 19%              | 84%                    |
| 141         | 350             | 211             | 139       | 40%              | 66%              | 60%                    |
| 142         | 730             | 463.5           | 266.5     | 37%              | 57%              | 63%                    |
| 143         | 490             | 231             | 259       | 53%              | 112%             | 47%                    |
| 144         | 160             | 87              | 73        | 46%              | 84%              | 54%                    |
| 145         | 380             | 222             | 158       | 42%              | 71%              | 58%                    |
| 146         | 210             | 93.5            | 116.5     | 55%              | 125%             | 45%                    |
| 147         | 630             | 383.4           | 246.6     | 39%              | 64%              | 61%                    |
| 148         | 150             | 83.2            | 66.8      | 45%              | 80%              | 55%                    |
| 149         | 320             | 152.5           | 167.5     | 52%              | 110%             | 48%                    |
| 150         | 900             | 467.6           | 432.4     | 48%              | 92%              | 52%                    |
| 151         | 1180            | 818.6           | 361.4     | 31%              | 44%              | 69%                    |
| 152         | 120             | 38.4            | 81.6      | 68%              | 213%             | 32%                    |

TABLE 1-continued

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 153         | 5230            | 1388            | 3842      | 73%              | 277%             | 27%                    |
| 154         | 34              | 10.5            | 23.5      | 69%              | 224%             | 31%                    |
| 155         | 1020            | 590.6           | 429.4     | 42%              | 73%              | 58%                    |
| 156         | 280             | 76.6            | 103.4     | 57%              | 135%             | 43%                    |
| 157         | 120             | 51.1            | 68.9      | 57%              | 135%             | 43%                    |
| Median      | 300             | 154             | 127       | 46%              | 84%              | 54%                    |

[0044] TABLE 2 shows the results, individually and comparatively, of the wPTH, PIN, and total PTH assays from the normals.

TABLE 2

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 1           | 17.13           | 3.32            | 13.81     | 81%              | 416%             | 19%                    |
| 2           | 32.92           | 10.49           | 22.43     | 68%              | 214%             | 32%                    |
| 3           | 31.32           | 10.31           | 21.01     | 67%              | 204%             | 33%                    |
| 4           | 41.84           | 12.72           | 29.12     | 70%              | 229%             | 30%                    |
| 5           | 33.03           | 10.09           | 22.94     | 69%              | 227%             | 31%                    |
| 6           | 44.32           | 14.23           | 30.09     | 68%              | 211%             | 32%                    |
| 7           | 31.47           | 6.80            | 24.67     | 78%              | 363%             | 22%                    |
| 8           | 20.82           | 10.03           | 10.79     | 52%              | 108%             | 48%                    |
| 9           | 34.64           | 15.95           | 18.69     | 54%              | 117%             | 46%                    |
| 10          | 23.69           | 5.25            | 18.44     | 78%              | 351%             | 22%                    |
| 11          | 53.98           | 17.82           | 36.16     | 67%              | 203%             | 33%                    |
| 12          | 52.71           | 18.83           | 33.88     | 64%              | 180%             | 36%                    |
| 13          | 26.92           | 5.63            | 21.29     | 79%              | 378%             | 21%                    |
| 14          | 39.93           | 11.86           | 28.07     | 70%              | 237%             | 30%                    |
| 15          | 48.84           | 20.47           | 28.37     | 58%              | 139%             | 42%                    |
| 16          | 29.56           | 13.68           | 15.88     | 54%              | 116%             | 46%                    |
| 17          | 36.19           | 14.69           | 21.50     | 59%              | 146%             | 41%                    |
| 18          | 20.96           | 6.99            | 13.97     | 67%              | 200%             | 33%                    |
| 19          | 59.29           | 27.89           | 31.40     | 53%              | 113%             | 47%                    |
| 20          | 45.57           | 18.23           | 27.34     | 60%              | 150%             | 40%                    |
| 21          | 35.64           | 18.72           | 16.92     | 47%              | 90%              | 53%                    |
| 22          | 38.53           | 19.56           | 18.97     | 49%              | 97%              | 51%                    |
| 23          | 21.71           | 9.34            | 12.37     | 57%              | 132%             | 43%                    |
| 24          | 32.42           | 13.51           | 18.91     | 58%              | 140%             | 42%                    |
| 25          | 28.50           | 10.41           | 18.09     | 63%              | 174%             | 37%                    |
| 26          | 18.17           | 7.80            | 10.37     | 57%              | 133%             | 43%                    |
| 27          | 39.96           | 17.29           | 22.67     | 57%              | 131%             | 43%                    |
| 28          | 34.08           | 15.24           | 18.84     | 55%              | 124%             | 45%                    |

TABLE 2-continued

| Patient No. | Total PTH pg/ml | Whole PTH pg/ml | PIN pg/ml | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|-------------|-----------------|-----------------|-----------|------------------|------------------|------------------------|
| 29          | 42.95           | 19.59           | 23.36     | 54%              | 119%             | 46%                    |
| 30          | 38.40           | 12.16           | 26.24     | 68%              | 216%             | 32%                    |
| 31          | 47.57           | 18.45           | 29.12     | 61%              | 158%             | 39%                    |
| MEDIAN      | 34.64           | 13.51           | 21.50     | 61%              | 158%             | 39%                    |

[0045] Clearly, the statistically significant differences in the medians of these two groups demonstrates that one can differentiate between the two by using these assays alone or by comparing their respective values.

TABLE 3

| Sample Type              | Total PTH (pg/mL) | Whole PTH (pg/mL) | PIN (pg/mL) | PIN to Total PTH | PIN to Whole PTH | Whole PTH to Total PTH |
|--------------------------|-------------------|-------------------|-------------|------------------|------------------|------------------------|
| Chronic uremia (n = 157) | 300               | 154               | 127         | 46%              | 84%              | 55%                    |
| Medians Normal (n = 31)  | 34.64             | 13.51             | 21.50       | 61%              | 158%             | 37%                    |
| Medians P-Value          | <0.0001           | <0.0001           | <0.0001     | <0.0001          | <0.0001          | <0.0001                |

[0046] The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

[0047] All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

[0048] Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.

SEQUENCE LISTING

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Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His  
 20 25 30

-continued

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Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser  
 35 40 45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu  
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Lys Ser Leu Gly Glu Ala Asn Lys Ala Asp Val Asn Val Leu Thr Lys  
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Ala Lys Ser Gln

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Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln Arg  
 35 40 45

Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser  
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Leu Gly Glu Ala Asn Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys  
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Ser Gln

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 <213> ORGANISM: Homo sapiens

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Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys  
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Ser Leu Gly Glu Ala Asn Lys Ala Asp Val Asn Val Leu Thr Lys Ala  
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Lys Ser Gln  
 50

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-continued

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<400> SEQUENCE: 6

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Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
                20                25                30

```

```

Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln Arg Pro Arg Lys Lys
                35                40                45

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Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser Leu Gly Glu Ala
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Asn Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
65                70                75

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<210> SEQ ID NO 7
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<400> SEQUENCE: 7

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1           5

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**1-46.** (canceled)

**47.** A method for determining the parathyroid hormone activity in a sample, comprising the steps:

reacting a sample with an antibody which recognizes an epitope in the region of the receptor binding structure of the parathyroid hormone;

reacting the sample with an antibody which recognizes an epitope that is formed of the N-terminal amino acids 1 to 4 of the parathyroid hormone and contains the N-terminal amino acids;

determining the number of molecules which are recognized by the two antibodies;

and/or calculating the effective parathyroid hormone activity from the number of the molecules in the sample.

**48.** The method according to claim 47, wherein the binding of the two antibodies is effected in the presence of a mild detergent.

**49.** The method according to claim 47, wherein one antibody is bound to a solid phase and the other antibody carries a marker.

**50.** The method according to claim 49, wherein the binding of the two antibodies is effected in the presence of a mild detergent.

**51.** The method of claim 47, wherein the effective parathyroid hormone activity and/or the determination of number of molecules is used in facilitating the diagnosis or assessment of the degree of hypo- or hyper-parathyroidism.

**52.** The method of claim 47, wherein the effective parathyroid hormone activity and/or the determination of number of molecules is used in determining a cause of disturbance to the calcium metabolism, osteopathies, kidney failure, or a disease which originates from a disturbed homeostasis of the calcium and phosphate contents of the blood.

**53.** A diagnosis system for determining parathyroid hormone activity in a sample, comprising:

an antibody which specifically recognizes an epitope formed of the N-terminal amino acids 1 to 4 of the parathyroid hormone and contains the N-terminal amino acids; and

an antibody which binds to the region of the receptor binding point of the parathyroid hormone.

\* \* \* \* \*

|                |  |         |            |
|----------------|--|---------|------------|
| 专利名称(译)        | 鉴别和监测甲状旁腺和骨骼状态相关疾病的方法  |         |            |
| 公开(公告)号        | <a href="#">US20050095631A1</a>  | 公开(公告)日 | 2005-05-05 |
| 申请号            | US10/945608  | 申请日     | 2004-09-20 |
| [标]申请(专利权)人(译) | 康托THOMAS大号<br>高平   |         |            |
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| 当前申请(专利权)人(译)  | Cantor THOMAS L.<br>GAO PING   |         |            |
| [标]发明人         | CANTOR THOMAS L<br>GAO PING  |         |            |
| 发明人            | CANTOR, THOMAS L.<br>GAO, PING   |         |            |
| IPC分类号         | G01N33/53 C07K14/635 C07K16/26 G01N33/68 G01N33/74 G01N33/78 C12Q1/68                      |         |            |
| CPC分类号         | C07K16/26 G01N33/6893 G01N33/74 C07K2317/34 G01N2800/046 G01N2800/10 Y10S436/811 G01N33/78 |         |            |
| 其他公开文献         | US7723042  |         |            |
| 外部链接           | <a href="#">Espacenet</a> <a href="#">USPTO</a>  |         |            |

摘要(译)

本发明涉及用于区分患者甲状旁腺疾病（例如甲状旁腺功能亢进和相关骨病）的正常或非疾病状态的新方法和装置。人们可以检测生物样本中的全部或非碎片（1至84）甲状旁腺激素，以及可以作为甲状旁腺激素拮抗剂起作用的大型非整体甲状旁腺激素肽片段。通过比较值或独立地使用大的非整体甲状旁腺激素肽片段，整个甲状旁腺激素或这些值的组合的值，能够区分甲状旁腺和骨相关疾病状态，以及区分这些状态来自正常状态。

FIG. 1

