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

VERFAHREN ZUM DIFFERENZIEREN UND ÜBERWACHEN VON PARATHYROID UND KRANKHEITEN, DIE MIT DEM ZUSTAND DER KNOCHEN VERBUNDEN SIND

PROCEDE POUR DIFFERENTIER ET SURVEILLER LES MALADIES LIEES A L'ETAT DE LA PARATHYROIDE ET DES OS

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

TECHNICAL FIELD

[0001] 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

[0002] 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 calcitrol 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.

[0003] 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 FIGURE 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-tenninal fragment and a (35-84) PTH C-temvnal 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)

[0004] 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.

[0005] 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 suppcession). 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 later 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.

[0006] Determining circulating biologically active PTH levels in humans has been challenging. One major problem is that PTH is found at low levels, normally 10pg/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).

[0007] PTH immunoassays have varied over the years. One early approach is a double antibody precipitation immunoassay found in U. S. 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.

[0008] 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, California. In one version, a capture antibody specifically binds to the C-terminal portion of hPTH while a labeled antibody specifically binds to the N-tetminal 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.

[0009] Recently, researchers have made a specific binding assay directed to the large N-terminal PTH fragments. (See. Gao, Ping *et alia "Immunochemicalluminometric 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.

[0010] MAEGERLEIN M ET AL: "A NEW IMMUNOENZYMOMETRIC ASSAY FOR BIOACTIVE N-TERMINAL HUMAN PARATHYROID HORMONE FRAGMENTS AND ITS APPLICATION IN PHARMACOKINETIC STUDIES IN DOGS", ARZNEIMITTEL FORSCHUNG. DRUG RESEARCH, EDITIO CANTOR. AULENDORF, DE, vol. 48, no. 2,1998, pages 199-204, XP001010446 ISSN: 0004-4172, discloses an assay for bioactive N-terminal parathyroid fragments which combines a monoclonal antibody (13C63/5) recognising hPTH fragment 16-24 with a polyclonal antibody (K2) showing a predominant binding sequence at hPTH 1-5.

[0011] In "Accumulation of a Non-(1-84) Molecular Form of Parathyroid Hormone (PTH) Detected by Intact PTH assay in Renal Failure: Importance in the Interpretation of PTH Values", BROSSARD ET AL., JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, 1996, p.3923-3929, intact PTH (I-PTH) and C-terminal PTH (C-PTH) were determined in normal and renal failure patients. C-PTH/1-PTH ratio is calculated and the relationship with parathyroid function was studied.

DISCLOSURE OF THE INVENTION

[0012] 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.

[0013] 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_{3-84}]) and (SEQ ID No. 3 (PTH_{34-84}]), functions *in vivo* as a wPTH antagonist or inhibitor (PIN), (see FIGURE 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.

[0014] 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, colorimetric 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.

[0015] 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 ofwPTH, 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, California. 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.

[0016] 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.

[0017] 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

[0018]

FIGURE 1 is a diagrammatic view of human wPTH.

FIGURE 2 is a diagrammatic view of a wPTH assay using the present antibody as a tracer element.

FIGURE 3 is a diagrammatic view of a wPTH assay using the present antibody as a capture element.

FIGURE 4 is a graph showing a standard curve for a wPTH assay.

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

FIGURE 6 is a diagrammatic view showing interference from PIN in conventional I-PTH assays.

FIGURE 7 is a graph comparing a conventional 1-PTH assay with the present wPTH assay for patients with chronic uremia.

FIGURE 8 is a graph showing the distribution ofwPTH values for healthy normal persons, patients with primary hyperparathyroidism, and patients with chronic uremia.

FIGURE 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.

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

FIGURE 11 is a graph demonstrating how the whole PTH assay used in the present invention does not detect to PIN.

FIGURE 12 is a graph demonstrating how PIN is an *in vivo* inhibitor of wPTH.

BEST MODES FOR CARRYING OUT THE INVENTION

[0019] 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 FIGURE 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

[0020] A preferred embodiment of the present invention is an immunoradiometric assay (IRMA), often referred to as a sandwich assay, as shown FIGURES 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 FIGURE 2. However, one could reverse the specificity of these antibodies, as is shown in FIGURE 3.

[0021] 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-tenminal 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 panicles.

[0022] 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-I radioisotope and reduction with sodium metabisulfate. Unincorporated 125-I radioisotope is separated from the 125-I-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 DPM (disintegrations per minute) per 100 microliters. This solution is the tracer solution to be used in the whole PTH IRMA.

[0023] Capture antibody coated tubes can be created by attaching affinity purified goat anti PTH 39-84 antibody, (Scantibodies Laboratory, Inc., Santee, California, U.S.A.), to 12 x 75 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.

[0024] 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 a computer software in order that the concentration of whole PTH in the samples may be ascertained. **FIGURE 4 shows a standard curve for such an assay.**

Initial whole PTH sequence peptide

[0025] In order to make the signal antibody in the above assay, first one makes a synthetic PTH peptide corresponding 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 be part of an affinity purification means for isolating the desired signal antibody or capture antibody.

[0026] Briefly, such a peptide can be synthesized on an Applied Biosystems, Inc. (Foster City, California, 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-blocked, 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, Massachusettts, U.S.A.) and reversed-phase HPLC using a VYDAC (TM) 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.

[0027] 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

[0028] To create an affinity-purified anti-(1-6) PTH antibody, one first uses a selected initial PTH sequence peptide as part of an immunogen for injection into a goat. The injectible immunogen is the wPTH complete peptidic sequence. The immunogen is mixed with an equal volume of Freunds complete adjuvant which is a mixture of light mineral oil, Arlacel 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

[0029] 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

[0030] The present w PTH 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, California, 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 FIGURE 10).

[0031] FIGURE 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 wPTH 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 FIGURE 11). FIGURE 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 FIGURE 6), but also can detect PIN, the large, non (1-84) PTH fragment, (as in the lower part of FIGURE 6).

[0032] A comparison of assay results for 157 chronic uremic patients is shown in FIGURE 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

[0033] 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 157 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.

[0034] Table 1 shows the results individually and comparatively, of the wPTH, PIN, and total PTH assays from chronic uremic patients on dialysis.

TABLE I

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%
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%

(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*
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%

(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*
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%
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%

(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*
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%

(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*
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%
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	180	76.6	103.4	57%	135%	43%
157	120	51.1	68.9	57%	135%	43%
Median	300	154	127	46%	84%	54%

[0035] 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	6.8	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	21.5	59%	146%	41%
18	20.96	6.99	13.97	67%	200%	33%
19	59.29	27.89	31.4	53%	113%	47%
20	45.57	18.23	27.34	60%	150%	40%
21	35.64	18.72	16.92	47%	90%	53%

(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*
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	28.5	10.41	18.09	63%	174%	37%
26	18.17	7.8	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%
29	42.95	19.59	23.36	54%	119%	46%
30	38.4	12.16	26.24	68%	216%	32%
31	47.57	18.45	29.12	61%	158%	39%
Median	34.64	13.51	21.5	61%	158%	39%

[0036] 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) Medians	300	154	127	46%	84%	55%
Normal (n**31) Medians	34.64	13.51	21.5	61%	158%	37%
P-Value	<00001	< 0.0001	<0.0001	< 0.0001	<0.0001	<0.0001

[0037] The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

SEQUENCE LISTING

[0038]

< 110> Cantor, Thomas L.
Gao, Ping

<120> Methods for Differentiating Parathyroid and Bone Status Related Diseases

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

Ala Gly Ser Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val
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20 25 30

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

Ser Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser
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His Glu Lys Ser Leu Gly Glu Ala Asn Lys Ala Asp Val Asn Val
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Leu Thyr Lys Ala Lys Ser Gln
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35 40 45

Thyr Lys Ala Lys Ser Gln
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Leu Gly

SEQUENCE LISTING

[0039]

<110> SCANTIBODIES LABORATORY INC

<120> METHODS FOR DIFFERENTIATING AND MONITORING PARATHYROID AND BONE STATUS RELATED DISEASES

<130> JL3016

<150> US 09/231,422
<151> 1999-01-14

<150> US09/344,639
<151> 1999-06-26

<150> PCT/US00/00855
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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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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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            20                  25                  30

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Lys Ser Gln
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<213> Homo sapiens

<400> 4

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<400> 5

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1               5                   10                  15

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

Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by**:

   a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen;

   b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

12. The method of claim 1, wherein the method is capable of detecting wPTH at a normal physiological level.

13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.

14. The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

15. The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

16. The method of claim 15, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

17. The method of claim 15 or 16, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

18. The method of claim 15 or 16, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

19. The method of claim 18, wherein the parathyroid disease is primary hyperparathyroidism.

20. The method of claim 18, wherein the parathyroid disease is secondary hyperparathyroidism.

21. The method of claim 18, wherein the parathyroid disease is caused by chronic renal failure.

22. The method of claim 18, wherein the parathyroid disease is renal osteodystrophy.

23. The method of claim 22, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

24. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

25. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

26. The method of Claim 18 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

27. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

28. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

29. The method of claim 28, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

30. The method of any of claims 15-17, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

31. The method of any of claims 18-30 wherein the comparison is in the form of a ratio or proportion.

32. The method of claim 18, wherein the sample is from a person who is a patient with chronic uremia.

33. The method of claim 31, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

35. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

36. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

37. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen.

38. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a monoclonal antibody.

39. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a polyclonal antibody.

40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of

detecting wPTH at a normal physiological level.

41. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.

42. The antibody or antibody fragment of any of claims 37-41, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), or a peptide of at least four amino acids in the common sequence.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch**:

a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
wodurch ein markierter Komplex gebildet wird; und
c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-

thormonantikörper ist.

12. Verfahren nach Anspruch 1, wobei das Verfahren dazu in der Lage ist, wPTH bei einem normalen physiologischen Spiegel zu detektieren.

13. Verfahren nach Anspruch 1, wobei das Verfahren in der Lage ist, wPTH bei Spiegeln von 27,89 pg/mL und niedriger zu detektieren.

14. Verfahren nach Anspruch 1, wobei die Probe ausgewählt ist aus der Gruppe, die aus einem Serum, einem Plasma und einer Blutprobe besteht.

15. Verfahren nach Anspruch 1, weiterhin umfassend den Schritt des Bestimmens entweder des Gesamtspiegels an Gesamt-PTH oder des Spiegels an Parathormon-Inhibitorpeptidfragment oder des Spiegels beider in der Probe.

16. Verfahren nach Anspruch 15, wobei der Spiegel ein Parathormon-Inhibitorpeptidfragment in der Probe durch Subtrahieren des gemessenen Spiegels an vollständigem PTH in der Probe vom gemessenen Spiegel an Gesamt-PTH in der Probe bestimmt wird, um den Spiegel an Parathormon-Inhibitorpeptidfragment zu berechnen.

17. verfahren nach Anspruch 15 oder 16, wobei der Gesamt-PTH-Spiegel unter Verwendung eines für das Fragment PTH_{7-38} spezifischen Antikörpers bestimmt wird.

18. Verfahren nach Anspruch 15 oder 16, weiterhin umfassend den Schritt des Vergleichens von zumindest zwei Parametern, die aus der Gruppe ausgewählt sind, die aus dem Spiegel an vollständigem Parathormon, dem Parathormon-Inhibitorpeptidfragmentspiegel und dem Gesamt-Parathormonspiegel besteht, wodurch bestimmt wird, ob die Probe von einer Person stammt, die im Wesentlichen normale Nebenschilddrüsenfunktion hat oder eine Nebenschilddrüsenerkrankung aufweist.

19. Verfahren nach Anspruch 18, wobei die Nebenschilddrüsenerkrankung primärer Hyperparathyroidismus ist.

20. Verfahren nach Anspruch 18, wobei die Nebenschilddrüsenerkrankung sekundärer Hyperparathyroidismus ist.

21. Verfahren nach Anspruch 18, wobei die Parathyroiderkrankung durch chronisches Nierenversagen verursacht wird.

22. Verfahren nach Anspruch 18, wobei die Nebenschilddrüsenerkrankung Nierenosteodystrophie ist.

23. Verfahren nach Anspruch 22, wobei die Nierenosteodystrophie ausgewählt ist aus der Gruppe, die aus Recklinghausen-Syndrom, Osteomalazie, extraskeletaler Verkalkung/Verknochung und einer adynamischen Knochenerkrankung besteht.

24. Verfahren nach Anspruch 18, wobei der Spiegel an vollständigem Parathormon mit dem Parathormon-Inhibotorpeptidfragmentspiegel verglichen wird.

25. Verfahren nach Anspruch 18, wobei der Spiegel an vollständigem Parathormon mit dem Gesamt-Parathormonspiegel in der Probe verglichen wird.

26. Verfahren nach Anspruch 18, wobei der Parathormon-Inhibitorpeptidfragmentspiegel mit dem Gesamt-Parathormonspiegel in der Probe verglichen wird.

27. Verfahren nach einem der Ansprüche 15 bis 17, weiterhin umfassend den Schritt des Vergleichens von zumindest zwei Parametern, die ausgewählt sind aus der Gruppe, die aus dem Spiegel an vollständigem Parathormon, dem Parathormon-Inhibitorpeptidfragmentspiegel und dem Gesamt-Parathormonspiegel besteht,
wodurch eine nebenschilddrüsenbezogene Knochenerkrankung und Behandlung in der Person, aus der die Probe genommen wurde, überwacht wird.

28. Verfahren nach einem der Ansprüche 15 bis 17, weiterhin umfassend den Schritt des Vergleichens von zumindest zwei Parametern, die ausgewählt sind aus der Gruppe, die aus dem Spiegel an vollständigem Parathormon, dem Parathormon-Inhibitorpeptidfragmentspiegel, und dem Gesamt-Parathormonspiegel besteht, wodurch Effekte der therapeutischen Behandlung des Hyperparathyroidismus in der Person, aus der die Probe genommen wurde, über-

wacht werden.

29. Verfahren nach Anspruch 28, wobei der Hyperparathyroidismus ausgewählt ist aus der Gruppe, die aus primärem Hyperparathyroidismus, sekundärem Hyperparathyroidismus, Nierenknochenerkrankung, Nierenosteodystrophie, Recklinghausen-Syndrom, Osteomalazie, extraskeletaler Verkalkung/Verknöcherung und einer adynamischen Knochenerkrankung besteht.

30. Verfahren nach einem der Ansprüche 15 bis 17, weiterhin umfassend den Schritt des Vergleichens des Spiegels an vollständigem Parathormon mit dem Parathormon-Inhibitorpeptidfragmentspiegel, um Nieren-Osteodystrophie und ihrer Behandlung zu überwachen.

31. Verfahren nach einem der Ansprüche 18 bis 30, wobei der Vergleich in Form eines Verhältnisses oder Anteils vorliegt.

32. verfahren nach Anspruch 18, wobei die Probe von einer Person stammt, die ein Patient mit chronischer Urämie ist.

33. Verfahren nach Anspruch 31, wobei das Parathormon-Inhibitorpeptidfragment ein Peptid mit einer Aminosäuresequenz des menschlichen PTH_{7-64} ist.

34. Verfahren nach Anspruch 15, wobei das Parathormon-Inhibitorpeptidfragment ein Peptid mit einer Aminosäuresequenz zwischen PTH_{3-84} (SEQ ID NO:2) und PTH_{34-84} (SEQ ID NO:3) ist und in vivo als Parathormonantagonist oder -inhibitor (PIN) wirkt.

35. Verfahren nach Anspruch 15, wobei das Parathormon-Inhibitorpeptidfragment ein Peptid mit einer Aminosäuresequenz des menschlichen PTH_{7-84} ist.

36. Verfahren nach Anspruch 1, weiterhin umfassend den Schritt der Verwendung des Spiegels an vollständigem Parathormon in der Probe zur Bestimmung, ob die Probe von einer Person stammt, die im Wesentlichen normale Parathyroidfunktion aufweist oder eine Parathyroiderkrankung aufweist.

37. Im Wesentlichen reiner/s Antikörper oder Antikörperfragment, das für eine Anfangspeptidsequenz von vollständigem Parathormon spezifisch ist, wobei die Anfangspeptidsequenz aus SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil von wPTH besteht, wobei zumindest vier Aminosäuren in der Sequenz Teile eines reaktiven Bereichs mit dem Antikörper oder Antikörperfragment sind, und wobei der/das Antikörper oder Antikörperfragment unter Verwendung der konkreten wPTH Peptid-Sequenz als Immunogen hergestellt wird.

38. Antikörper oder Antikörperfragment nach Anspruch 37, wobei der/das Antikörper oder Antikörperfragment ein monoklonaler Antikörper ist.

39. Antikörper oder Antikörperfragment nach Anspruch 37, wobei der/das Antikörper oder Antikörperfragment ein polyklonaler Antikörper ist.

40. Antikörper oder Antikörperfragment nach einem der Ansprüche 37 bis 39, wobei der/das Antikörper oder Antikörperfragment dazu in der Lage ist, wPTH bei normalen physiologischen Spiegeln zu detektieren.

41. Antikörper oder Antikörperfragment nach einem der Ansprüche 37 bis 39, wobei der/das Antikörper oder Antikörperfragment zum Detektieren von wPTH bei Spiegeln von 27,89 pg/ml und niedriger in der Lage ist.

42. Antikörper oder Antikörperfragment eines der Ansprüche 37 bis 41 oder das Verfahren eines der Ansprüche 1 bis 36, wobei der/das erste Antikörper oder Antikörperfragment unter Verwendung eines synthetischen Peptids affinitätsgereinigt wird, das ausgewählt ist aus hPTH 1-8(SER-VAL-SER-GLU-ILE-GLN-LEU-MET), fat-PTH 1-8(ALA-VAL-SER-GLU-ILE-GLN-LEU-MET) und einem Peptid von zumindest vier Aminosäuren in der gemeinsamen Sequenz.

Revendications

1. Procédé de mesure de la quantité d'hormone parathyroïdienne entière dans un échantillon sans détection d'un fragment d'hormone parathyroïdienne interférant non- (1-84), ledit procédé étant **caractérisé par** :

a) l'addition à l'échantillon d'un premier anticorps ou fragment d'anticorps spécifique du peptide d'hormone parathyroïdienne SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) qui fait partie de la séquence complète d'hormone parathyroïdienne entière (wPTH), et dans lequel au moins quatre acides aminés dans ledit peptide font partie d'une portion réactive au dit premier anticorps ou fragment d'anticorps; lequel premier anticorps ou fragment d'anticorps est produit en utilisant la séquence peptidique complète de wPTH comme immunogène,
b) l'addition d'un second anticorps ou fragment d'anticorps qui se lie spécifiquement à une portion de l'hormone parathyroïdienne entière différente de la séquence peptidique d'hormone parathyroïdienne initiale qui se lie au premier anticorps,
dans lequel, l'un ou l'autre du premier anticorps ou fragment d'anticorps ou du second anticorps ou fragment d'anticorps est marqué,
en formant de cette façon un complexe marqué ; et
c) la mesure de la quantité du complexe marqué pour mesurer la quantité d'hormone parathyroïdienne entière dans l'échantillon.

2. Procédé selon la revendication 1, dans lequel le second anticorps ou fragment d'anticorps est ajouté séquentiellement ou simultanément avec le premier anticorps ou fragment d'anticorps.

3. Procédé selon la revendication 1, dans lequel le premier anticorps ou fragment d'anticorps est lié à un support solide.

4. Procédé selon la revendication 3, dans lequel le premier anticorps ou fragment d'anticorps est lié à un support solide colloïdal.

5. Procédé selon la revendication 4, dans lequel le support colloïdal est sous la forme de particules de latex.

6. Procédé selon la revendication 1, dans lequel le premier anticorps ou fragment d'anticorps est marqué et est un anticorps monoclonal.

7. Procédé selon la revendication 1, dans lequel le premier anticorps ou fragment d'anticorps est marqué et est un anticorps polyclonal.

8. Procédé selon la revendication 1, dans lequel le second anticorps ou fragment d'anticorps est marqué.

9. Procédé selon la revendication 1, dans lequel le second anticorps ou fragment d'anticorps est lié à un support solide.

10. Procédé selon la revendication 1, dans lequel le marqueur de l'anticorps ou du fragment d'anticorps marqué est sélectionné dans l'ensemble constitué d'un agent chimioluminescent, d'un agent colorimétrique, d'un agent de transfert d'énergie, d'un enzyme, d'un agent fluorescent et d'un isotope radioactif.

11. Procédé selon la revendication 1, dans lequel le premier anticorps ou fragment d'anticorps est un anticorps anti-hormone parathyroïdienne (1-6) de chèvre.

12. Procédé selon la revendication 1, dans lequel le procédé est capable de détecter wPTH à un niveau physiologique normal.

13. Procédé selon la revendication 1, dans lequel le procédé est capable de détecter wPTH à des niveaux inférieurs ou égaux à 27,89 pg/ml.

14. Procédé selon la revendication 1, dans lequel l'échantillon est sélectionné dans le groupe constitué d'un échantillon de sérum, de plasma et de sang.

15. Procédé selon la revendication 1, comprenant en outre l'étape de détermination du niveau de PTH total ou du niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne ou du niveau des deux dans l'échantillon.

16. Procédé selon la revendication 15, dans lequel le niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne dans l'échantillon est déterminé en soustrayant le niveau mesuré de PTH entière dans l'échantillon du niveau mesuré total de PTH dans l'échantillon pour calculer le niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne.

17. Procédé selon la revendication 15 ou 16, dans lequel le niveau total de PTH est déterminé en utilisant un anticorps spécifique du fragment PTH_{7-38}.

18. Procédé selon la revendication 15 ou 16, comprenant en outre l'étape de comparaison d'au moins deux paramètres sélectionnés dans l'ensemble constitué du niveau de l'hormone parathyroïdienne entière, du niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne et du niveau total en hormone parathyroïdienne,
en déterminant de cette façon si l'échantillon provient d'une personne qui a une fonction parathyroïdienne essentiellement normale ou a une maladie parathyroïdienne.

19. Procédé selon la revendication 18, dans lequel la maladie parathyroïdienne est une hyperparathyroïdie primitive.

20. Procédé selon la revendication 18, dans lequel la maladie parathyroïdienne est une hyperparathyroïdie secondaire.

21. Procédé selon la revendication 18, dans lequel la maladie parathyroïdienne est provoquée par une insuffisance rénale chronique.

22. Procédé selon la revendication 18, dans lequel la maladie parathyroïdienne est une ostéodystrophie rénale.

23. Procédé selon la revendication 22, dans lequel l'ostéodystrophie rénale est sélectionnée dans l'ensemble constitué d'une ostéite fibro-kystique, d'une ostéomalacie, d'une calcification/ossification extrasquelettique et d'une maladie osseuse adynamique.

24. Procédé selon la revendication 18, dans lequel le niveau de l'hormone parathyroïdienne entière est comparé avec le niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne.

25. Procédé selon la revendication 18, dans lequel le niveau de l'hormone parathyroïdienne entière est comparé avec le niveau total de l'hormone parathyroïdienne dans l'échantillon.

26. Procédé selon la revendication 18, dans lequel le niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne est comparé avec le niveau total de l'hormone parathyroïdienne dans l'échantillon.

27. Procédé selon l'une quelconque des revendications 15 à 17, comprenant en outre l'étape consistant à comparer au moins deux paramètres sélectionnés dans l'ensemble constitué du niveau de l'hormone parathyroïdienne entière, du niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne et du niveau total de l'hormone parathyroïdienne,
en contrôlant de cette façon la maladie osseuse liée à la parathyroïde et le traitement de la personne à partir de laquelle l'échantillon a été collecté.

28. Procédé selon l'une quelconque des revendications 15 à 17, comprenant en outre l'étape de comparaison d'au moins deux paramètres sélectionnés dans l'ensemble constitué du niveau de l'hormone parathyroïdienne entière, du niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne et du niveau total de l'hormone parathyroïdienne, en contrôlant de cette façon les effets du traitement thérapeutique de l'hyperparathyroïdie chez la personne à partir de laquelle l'échantillon a été collecté.

29. Procédé selon la revendication 28, dans lequel l'hyperparathyroïdie est sélectionnée dans l'ensemble constitué de l'hyperparathyroïdie primitive, de l'hyperparathyroïdie secondaire, d'une maladie osseuse rénale, d'une ostéodystrophie rénale, d'une ostéite fibro-kystique, d'une ostéomalacie, d'une calcification/ossification extra-squelettique et d'une maladie osseuse adynamique.

30. Procédé selon l'une quelconque des revendications 15 à 17, comprenant en outre, l'étape consistant à comparer le niveau de l'hormone parathyroïdienne entière avec le niveau du fragment peptidique inhibiteur d'hormone parathyroïdienne, pour contrôler l'ostéodystrophie rénale et son traitement.

31. Procédé selon l'une quelconque des revendications 18 à 30, dans lequel la comparaison est sous forme d'un rapport ou d'une proportion.

32. Procédé selon la revendication 18, dans lequel l'échantillon provient d'une personne qui est un patient atteint d'urémie chronique.

33. Procédé selon la revendication 31, dans lequel le fragment peptidique inhibiteur d'hormone parathyroïdienne est un peptide ayant une séquence d'acides aminés de PTH_{7-84}, humain.

34. Procédé selon la revendication 15, dans lequel le fragment peptidique inhibiteur d'hormone parathyroïdienne est un peptide ayant une séquence d'acides aminés d'entre PTH_{3-84} (SEQ ID NO:2) et PTH_{34-84} (SEQ ID NO:3) et fonctionne *in vivo* comme un antagoniste ou un inhibiteur d'hormone parathyroïdienne (PIN).

35. Procédé selon la revendication 15, dans lequel le fragment peptidique inhibiteur d'hormone parathyroïdienne est un peptide ayant une séquence d'acides aminés de PTH_{7-84} humain.

36. Procédé selon la revendication 1, comprenant en outre l'étape d'utilisation du niveau d'hormone parathyroïdienne entière dans l'échantillon pour déterminer si l'échantillon provient d'une personne qui a une fonction parathyroïdienne essentiellement normale ou a une maladie parathyroïdienne.

37. Anticorps ou fragment d'anticorps essentiellement pur spécifique d'une séquence peptidique initiale d'hormone parathyroïdienne entière, dans lequel la séquence peptidique initiale consiste en SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) qui fait partie de wPTH, dans laquelle au moins quatre acides aminés dans cette séquence font partie d'une portion réactive à l'anticorps ou le fragment d'anticorps, et dans lequel l'anticorps ou fragment d'anticorps est produit en utilisant la séquence peptidique complète de wPTH comme immunogène.

38. Anticorps ou fragment d'anticorps selon la revendication 37, dans lequel l'anticorps ou fragment d'anticorps est un anticorps monoclonal.

39. Anticorps ou fragment d'anticorps selon la revendication 37, dans lequel l'anticorps ou fragment d'anticorps est un anticorps polyclonal.

40. Anticorps ou fragment d'anticorps selon l'une quelconque des revendications 37 à 39, dans lequel l'anticorps ou fragment d'anticorps est capable de détecter wPTH à un niveau physiologique normal.

41. Anticorps ou fragment d'anticorps selon l'une quelconque des revendications 37 à 39, dans lequel l'anticorps ou fragment d'anticorps est capable de détecter wPTH à des niveaux inférieurs ou égaux à 27,89 pg/ml.

42. Anticorps ou fragment d'anticorps selon l'une quelconque des revendications 37 à 41 ou procédé selon l'une quelconque des revendications 1 à 36, dans lequel le premier anticorps ou fragment d'anticorps est purifié par affinité en utilisant un peptide synthétique sélectionné parmi hPTH 1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), PTH 1-8 de rat (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)) et un peptide d'au moins quatre acides aminés dans la séquence commune.

FIG. 1

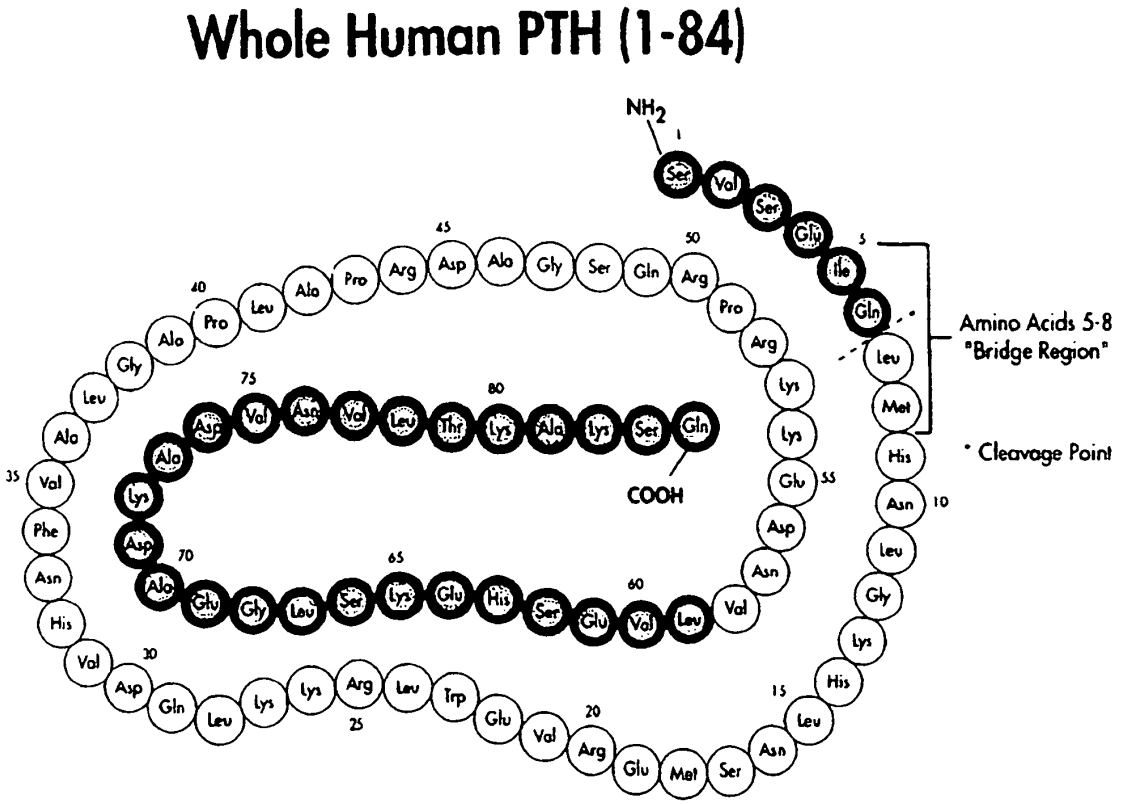

Whole Human PTH (1-84)
NH2
COOH
Amino Acids 5-8
"Bridge Region"
* Cleavage Point

FIG. 2

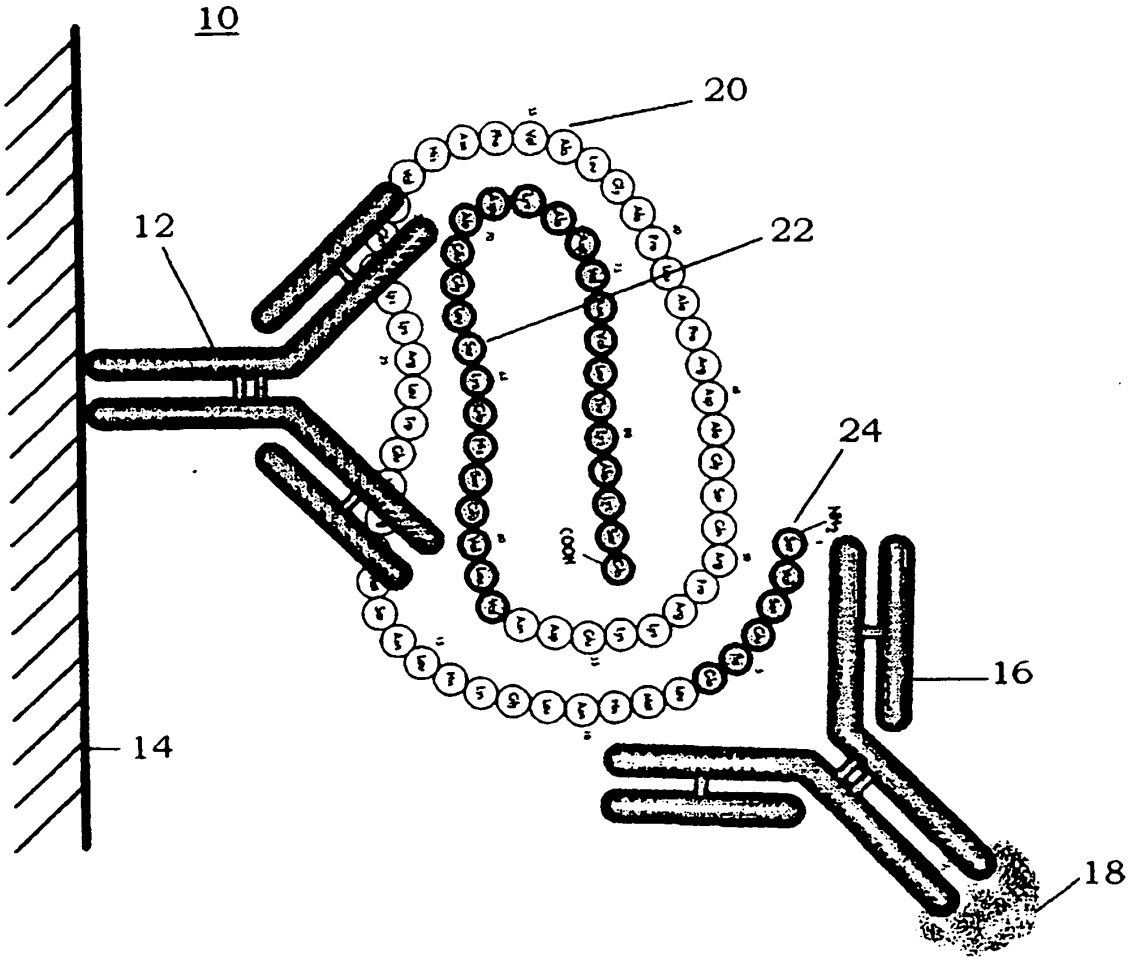


FIG. 3

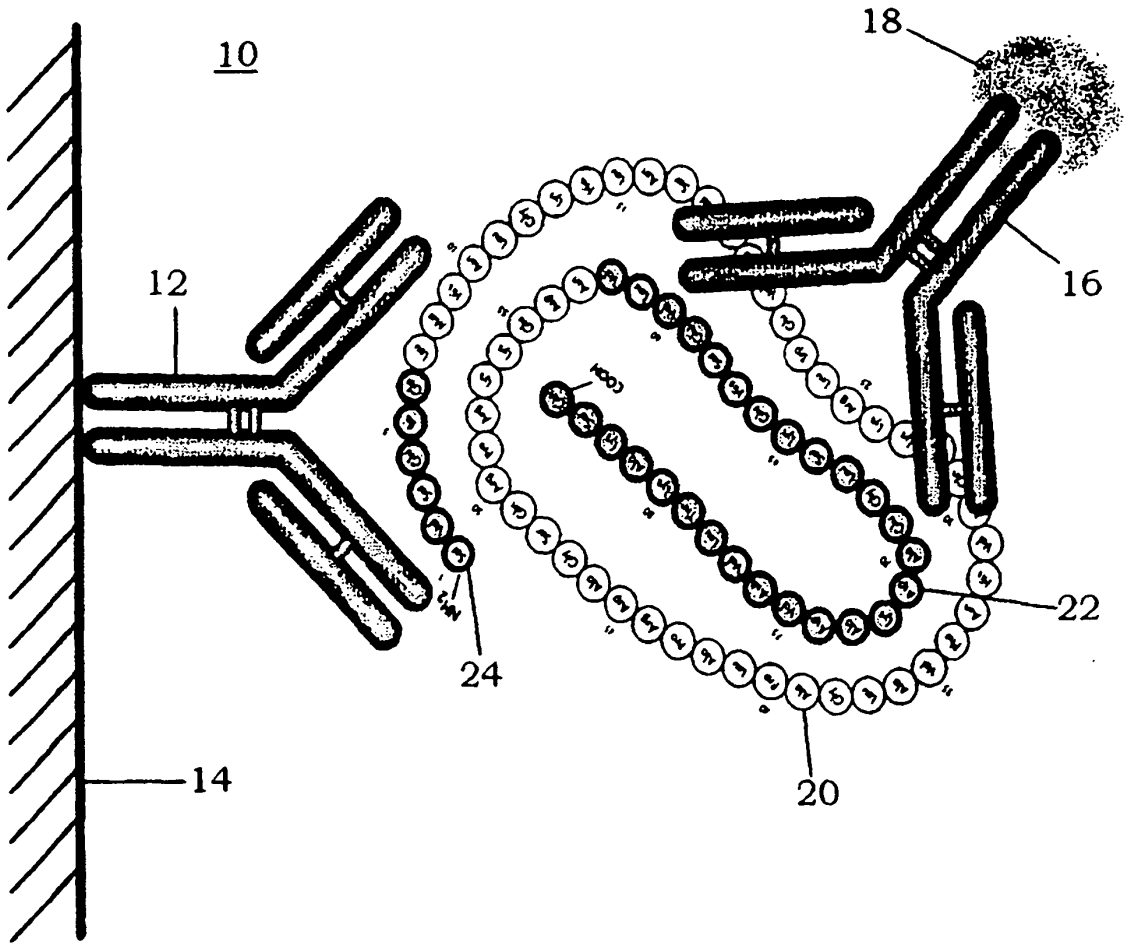


FIG. 4

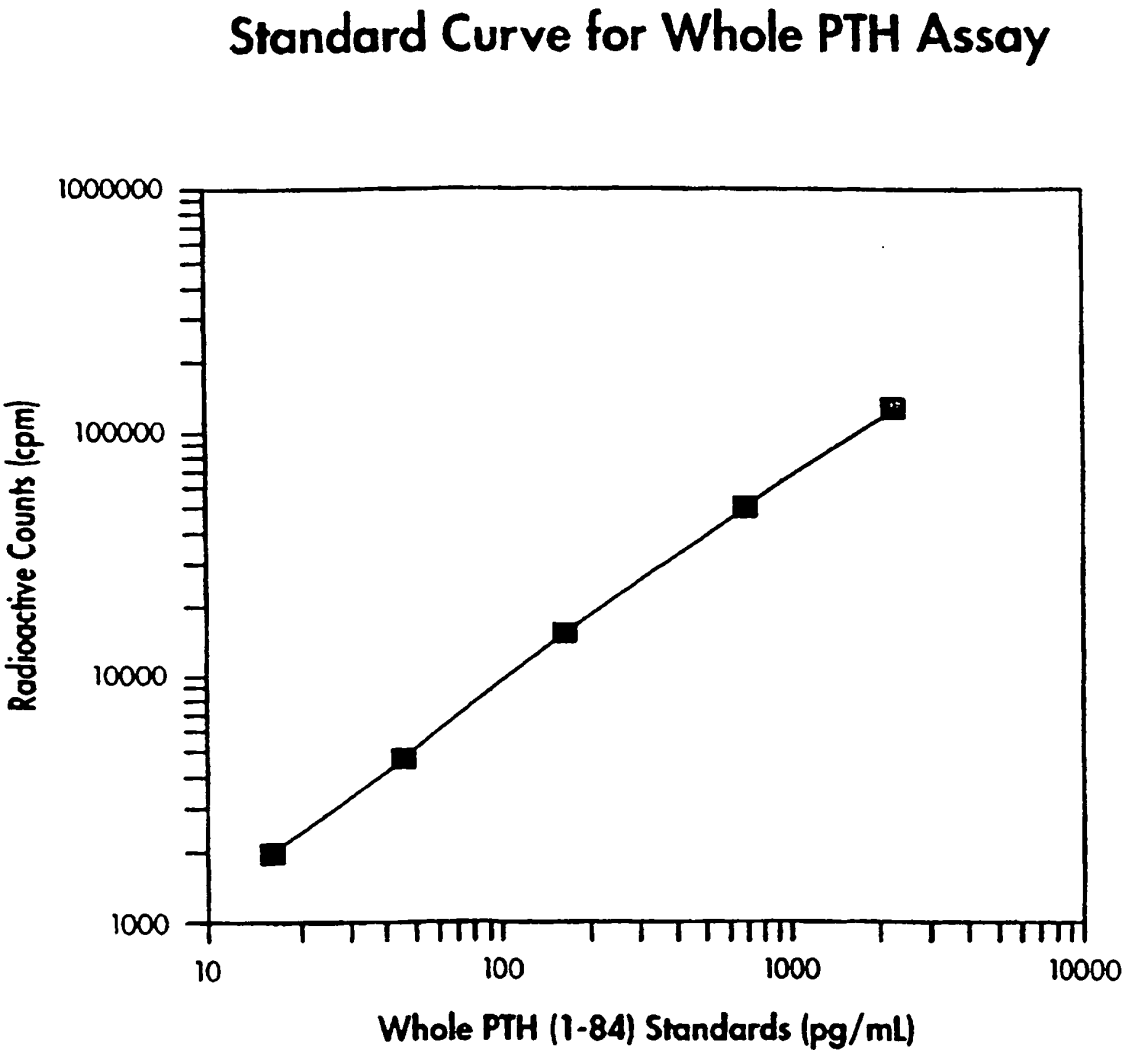

Standard Curve for Whole PTH Assay
1000000
100000
10000
1000
Radioactive Counts (cpm)
10
100
1000
10000
Whole PTH (1-84) Standards (pg/mL)

FIG. 5

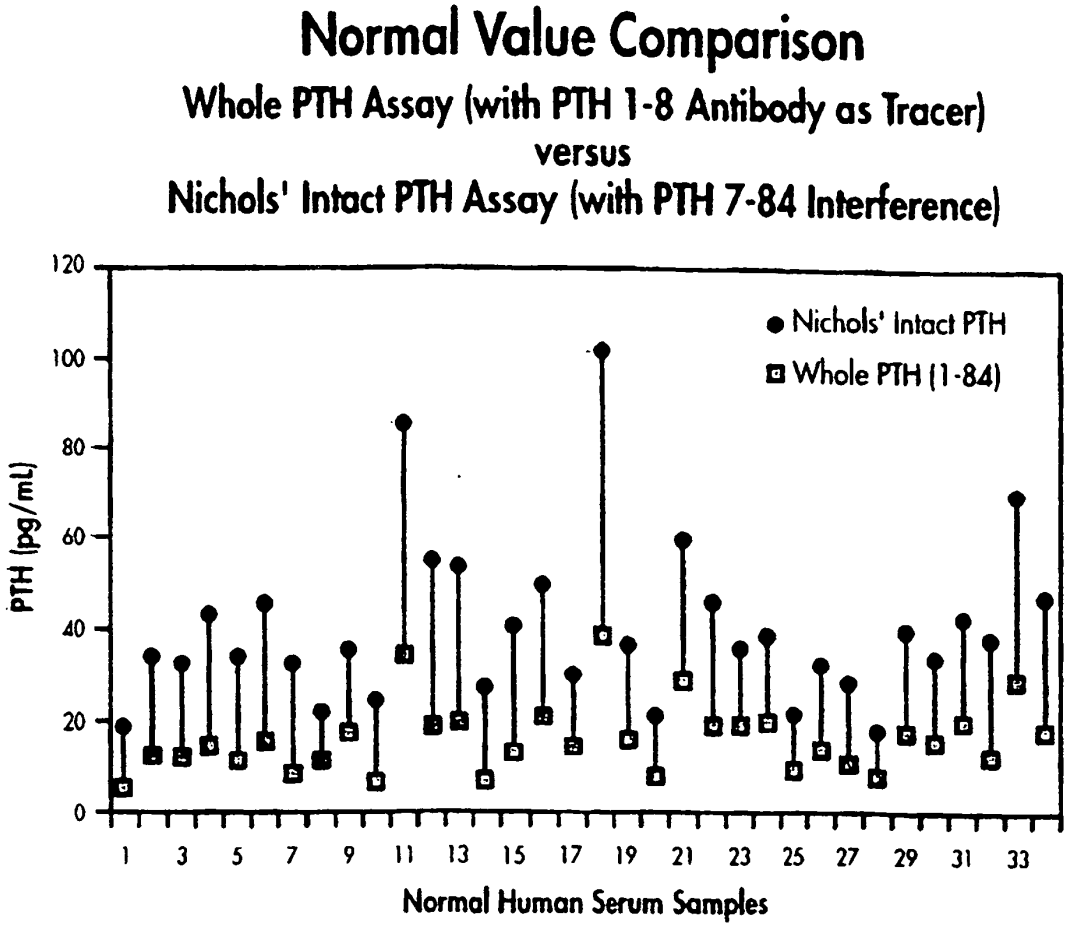

Normal Value Comparison
Whole PTH Assay (with PTH 1-8 Antibody as Tracer)
versus
Nichols' Intact PTH Assay (with PTH 7-84 Interference)
Nichols' Intact PTH
Whole PTH (1-84)
PTH (pg/mL)
120
100
80
60
40
20
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
Normal Human Serum Samples

FIG. 6

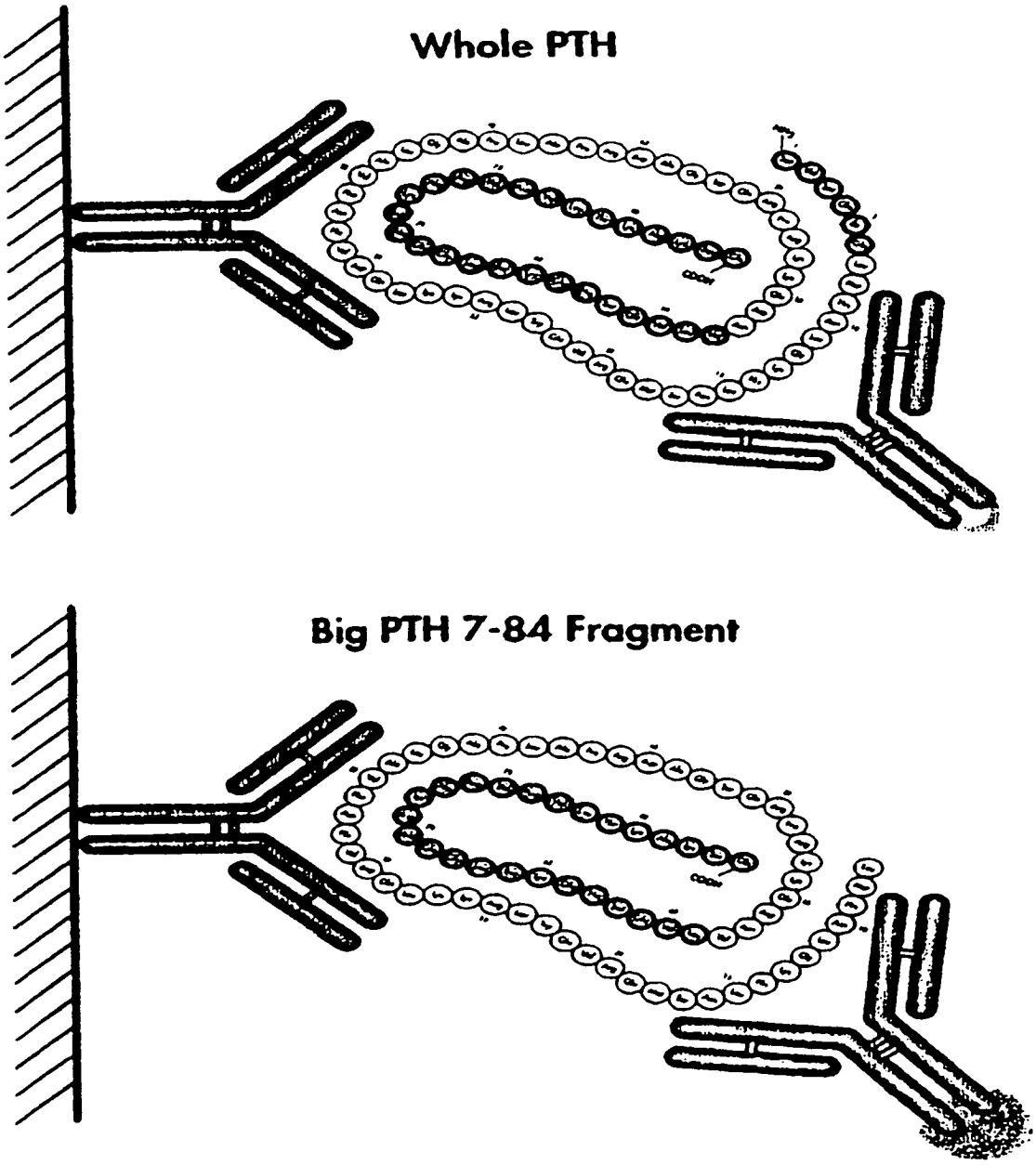


FIG. 7

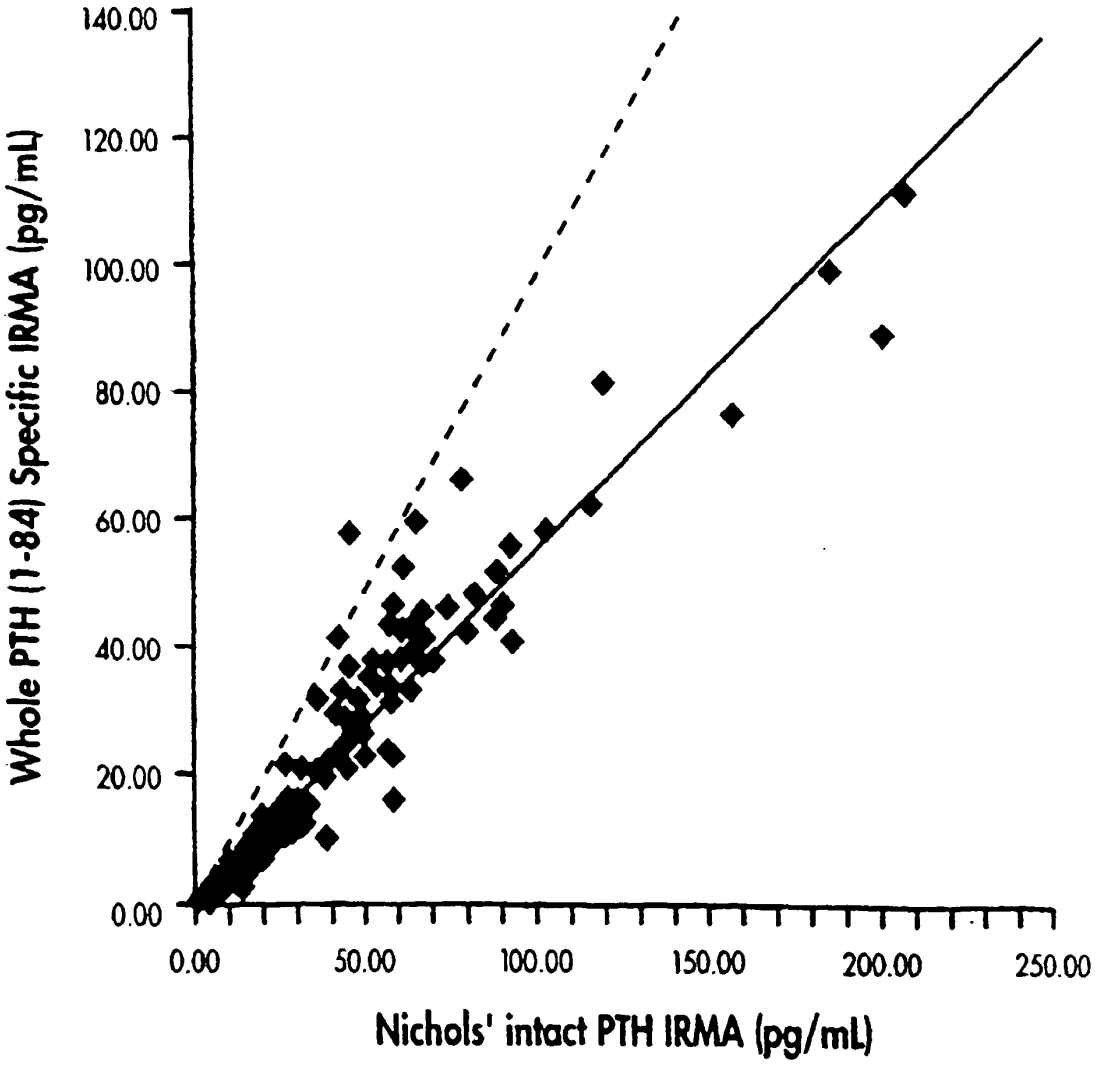

140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Whole PTH (1-84) Specific IRMA (pg/mL)
0.00
50.00
100.00
150.00
200.00
250.00
Nichols' intact PTH IRMA (pg/mL)

FIG. 8

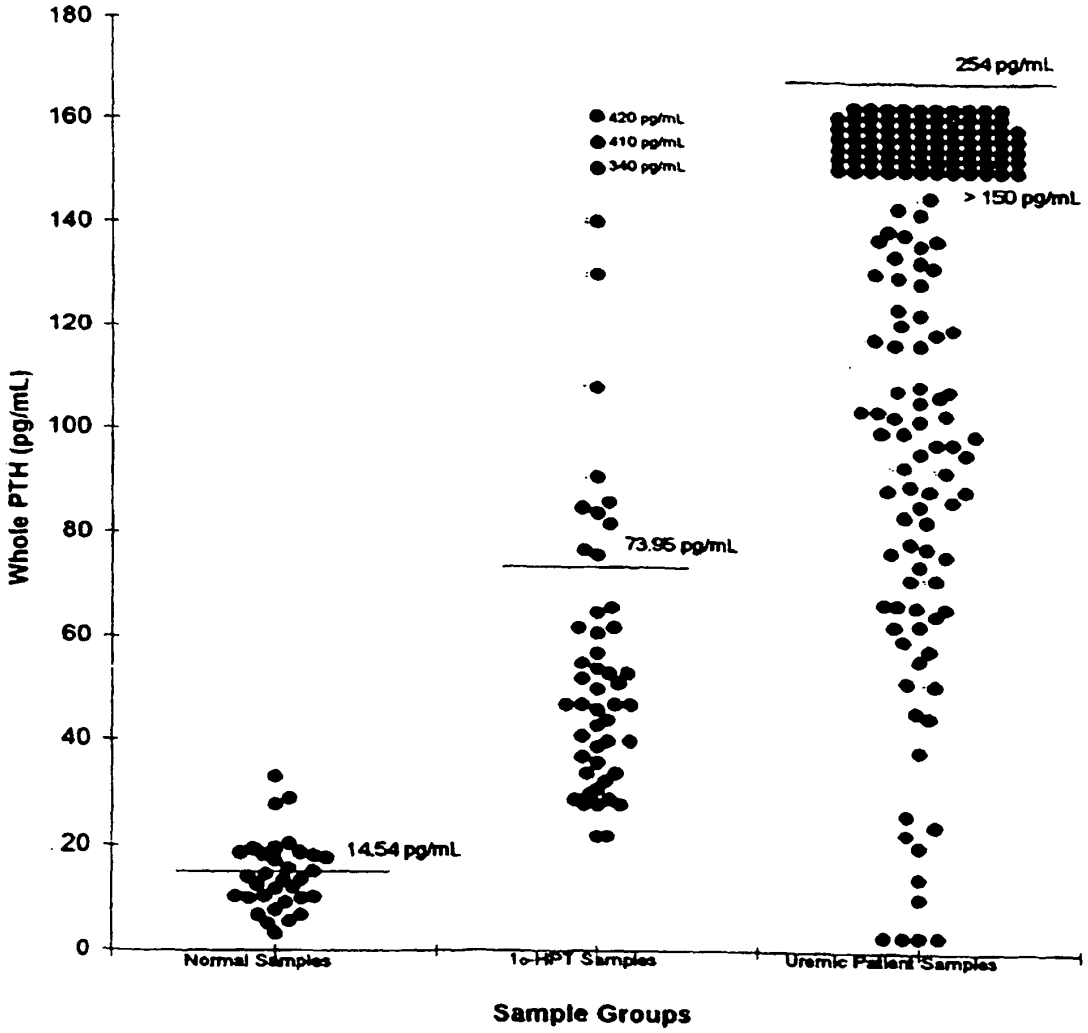

180
160
140
120
100
80
60
40
20
0
Whole PTH (pg/mL)
254 pg/mL
420 pg/mL
410 pg/mL
340 pg/mL
> 150 pg/mL
73.95 pg/mL
14.54 pg/mL
Normal Samples
1o-HPT Samples
Uremic Patient Samples
Sample Groups

FIG. 9

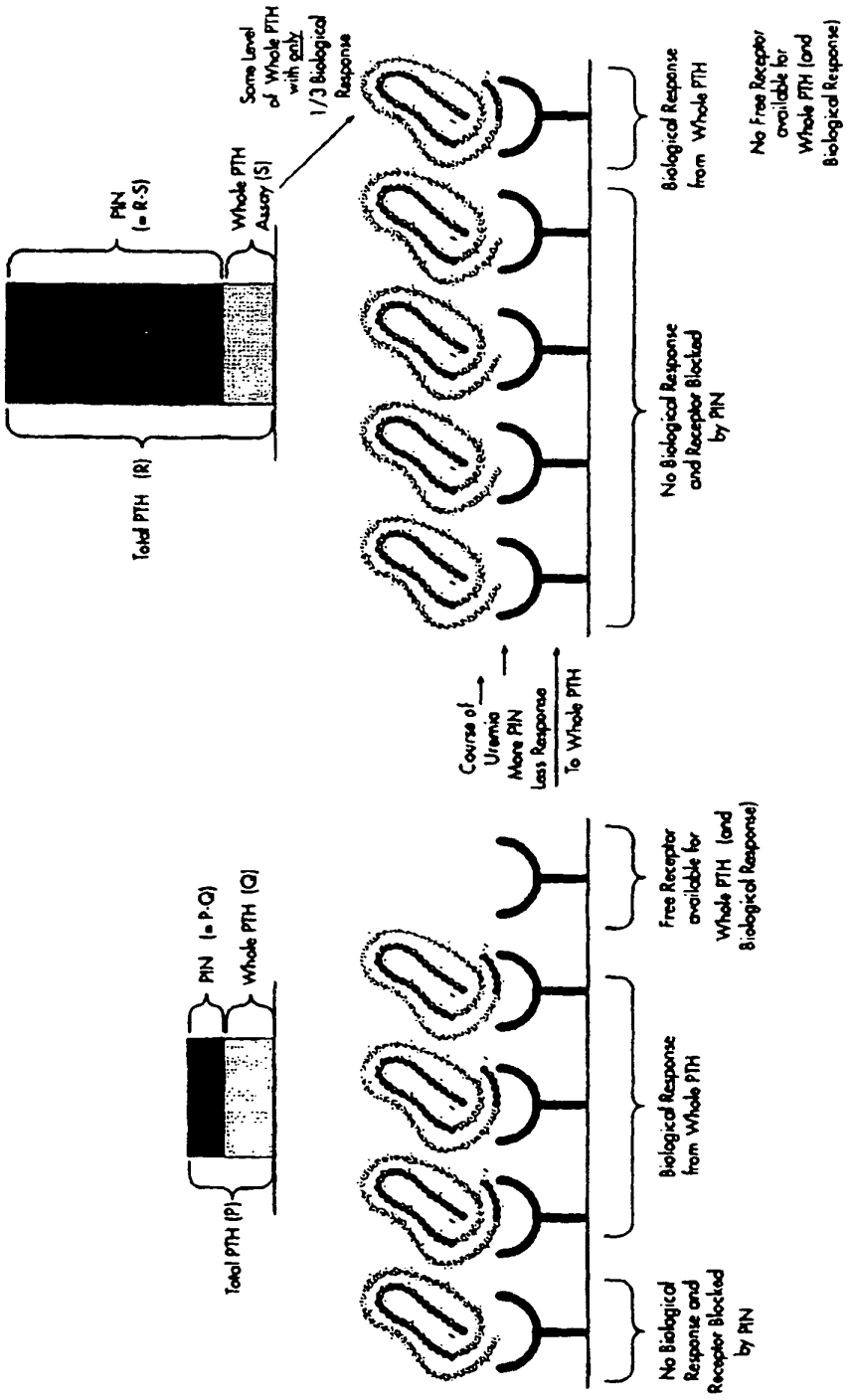

PIN (= P-Q)
Whole PTH (Q)
Total PTH (P)
PIN (= R-S)
Whole PTH Assay (S)
Total PTH (R)
Some Level of Whole PTH with only 1/3 Biological Response
Course of Uremia
More PIN
Less Response
To Whole PTH
No Biological Response and Receptor Blocked by PIN
Biological Response from Whole PTH
Free Receptor available for Whole PTH (and Biological Response)
No Biological Response and Receptor Blocked by PIN
Biological Response from Whole PTH
No Free Receptor available for Whole PTH (and Biological Response)

FIG. 10

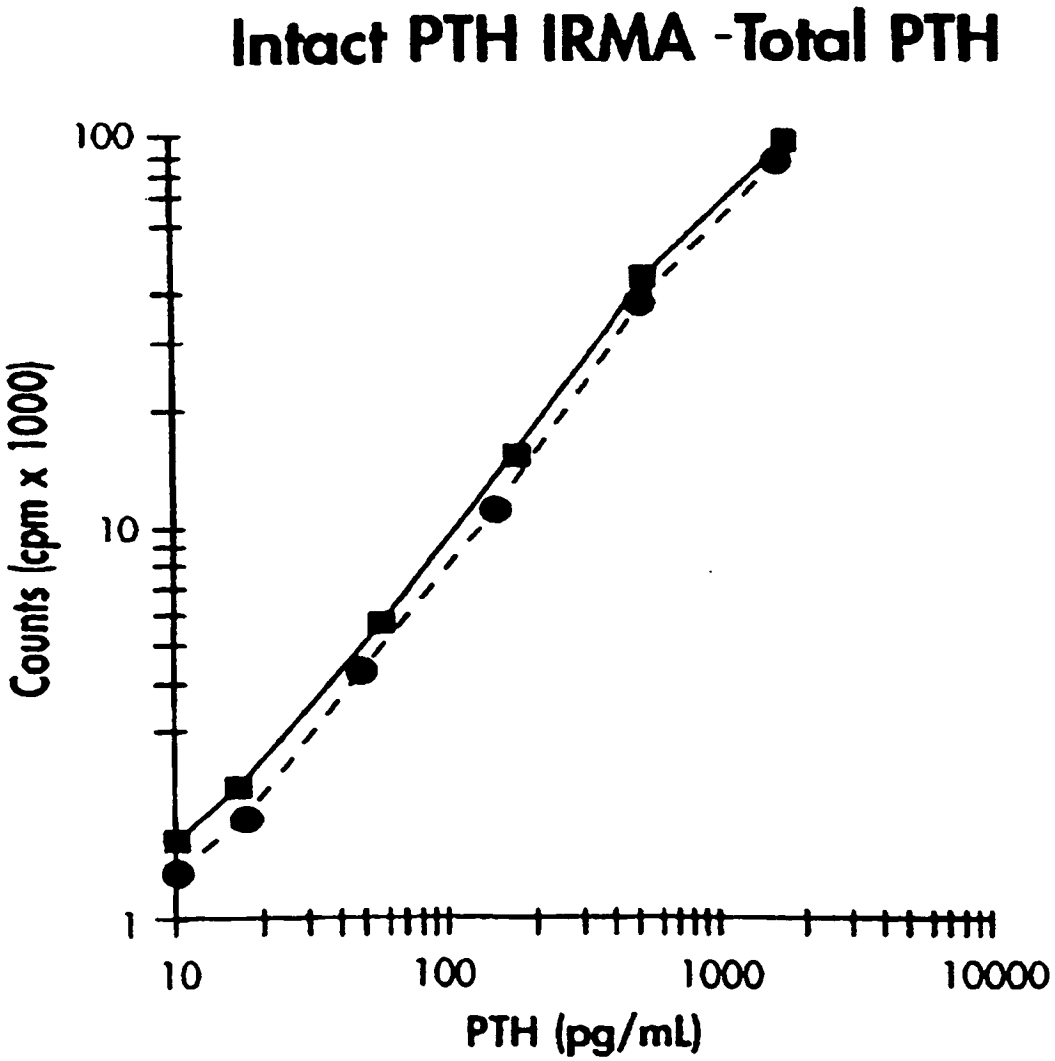

Intact PTH IRMA -Total PTH
Counts (cpm x 1000)
100
10
1
10
100
1000
10000
PTH (pg/mL)

FIG. 11

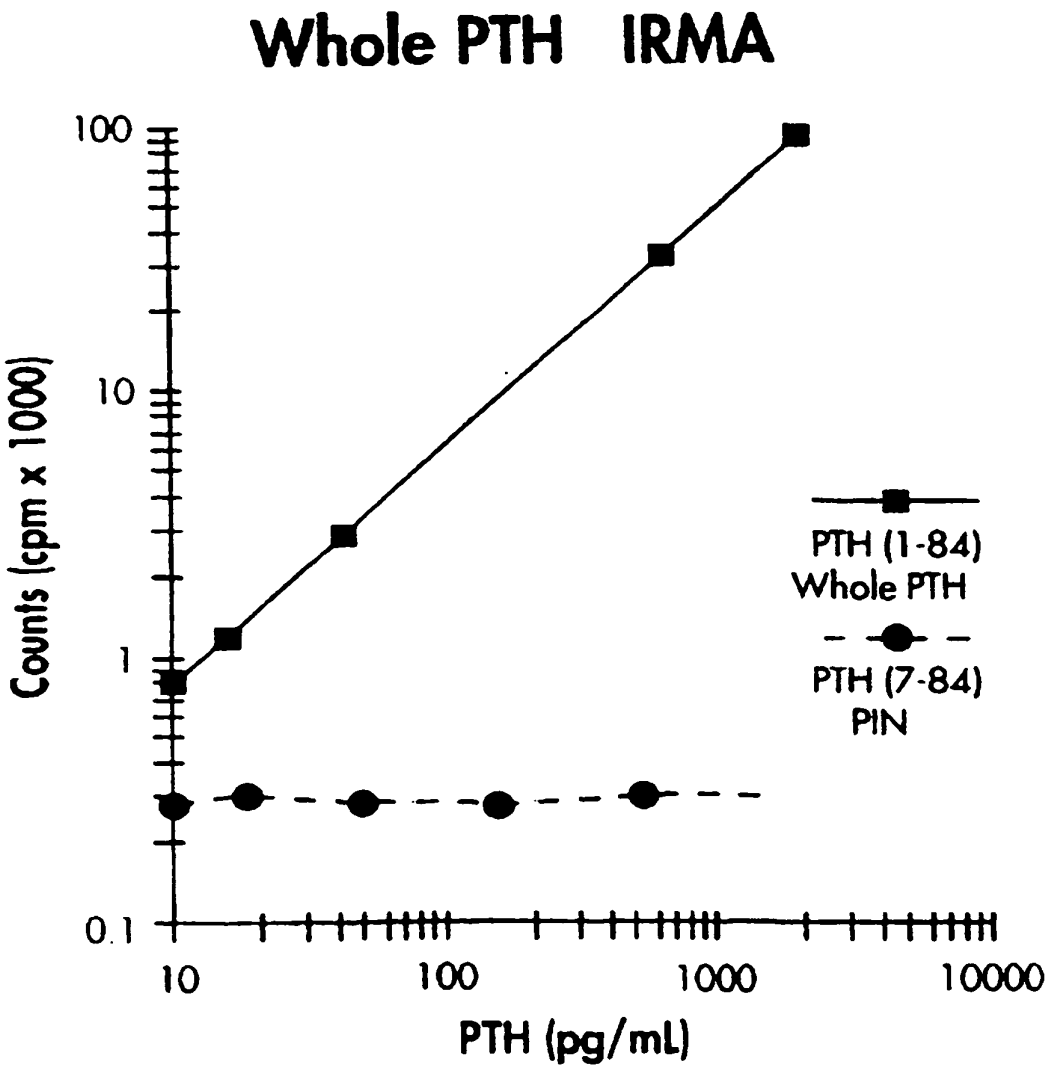
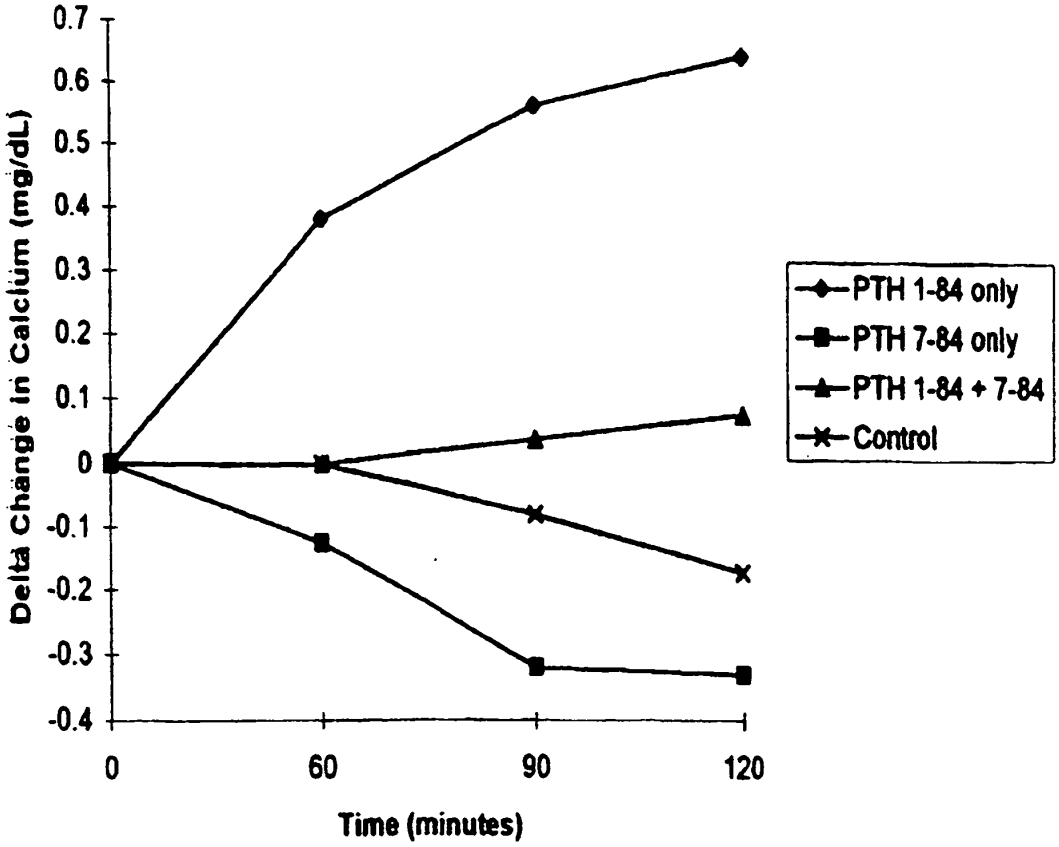

Whole PTH IRMA
100
10
1
0.1
Counts (cpm x 1000)
10
100
1000
10000
PTH (pg/mL)
PTH (1-84)
Whole PTH
PTH (7-84)
PIN

FIG. 12


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
Delta Change in Calcium (mg/dL)
0
60
90
120
Time (minutes)
PTH 1-84 only
PTH 7-84 only
PTH 1-84 + 7-84
Control

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		
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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。

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu
1 5 10 15

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

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

Ala Gly Ser Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val
50 55 60