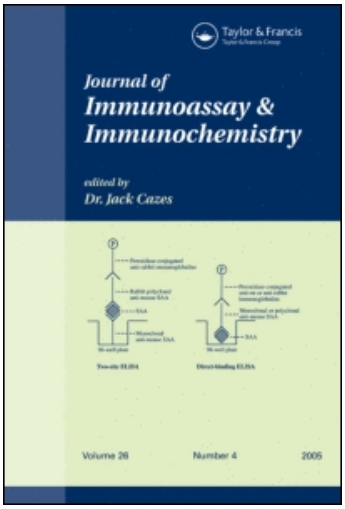

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Parathyroid Hormone Radioimmunoassay: The Clinical Evaluation of Assays Using Commercially Available Reagents

Graeme K. Worth[a]; Geoffrey C. Nicholson[a]; Robert W. Retallack[a]; Donald H. Gutteridge[a]; Jacqueline C. Kent[a]

[a] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia

To cite this Article Worth, Graeme K. , Nicholson, Geoffrey C. , Retallack, Robert W. , Gutteridge, Donald H. and Kent, Jacqueline C.(1985) 'Parathyroid Hormone Radioimmunoassay: The Clinical Evaluation of Assays Using Commercially Available Reagents', Journal of Immunoassay and Immunochemistry, 6: 3, 277 — 298

To link to this Article: DOI: 10.1080/01971528508063034

URL: http://dx.doi.org/10.1080/01971528508063034

JOURNAL OF IMMUNOASSAY, 6(3), 277-298 (1985)

PARATHYROID HORMONE RADIOIMMUNOASSAY: THE CLINICAL EVALUATION OF ASSAYS USING COMMERCIALLY AVAILABLE REAGENTS

Graeme K. Worth, Geoffrey C. Nicholson, Robert W. Retallack, Donald H. Gutteridge and Jacqueline C. Kent. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Verdun Street, Nedlands, Western Australia

(KEY WORDS: PTH RIA, Hyperparathyroidism, malignancy, hypercalcaemia.)

ABSTRACT

This paper reports on the diagnostic usefulness of two commercial PTH assay kits and four "in-house" assays using commercially available reagents, studying the same samples from normal controls and different patient groups. The ability of such assays to discriminate proven primary hyperparathyroid (1° HPT) patients from normals varied significantly but without any apparent correlation with assay components. For all assays, performance declined markedly in 1° HPT patient groups with lower serum calcium levels. Patients with PTH secondary to chronic renal disease were well discriminated from normal by all assays. Although immunoassays are useful in many cases of 1° HPT, it is difficult to develop C-terminal or mid-region PTH assays that are uniformly diagnostically useful in the clinical situation where they are of greatest potential use i.e. in cases of mildly hypercalcaemic 1° HPT.

INTRODUCTION

The numerous problems inherent in parathyroid hormone (PTH) radioimmunoassay (RIA) have been discussed elsewhere (eg 1,2,3). One suggestion to overcome these problems has been to make the assay system as homologous as possible, ideally by the use of

 0197-1522/85/0603-0277$3.50/0

human PTH (hPTH) for label, standard and antigen, although the limited availability of hPTH precludes the widespread realisation of this objective. A number of assays using synthetic hPTH fragments have been reported, directed towards either the C-terminus (eg 4), mid-region (eg 5,6,7,8,9) or less frequently the N-terminus (eg 10). However most commercial kits and many "in-house" assays still use components derived from bovine PTH (bPTH). It is generally agreed (1,2,3,11) that for routine clinical purposes N-terminal assays are of lesser importance, and will not be discussed in this paper. It has been suggested (7,8,9,12) that mid-region assays detect in hyperparathyroid sera a large fragment, presumably lacking both the N-terminus and the extreme C-terminus, that is not detected by "most" C-terminal assays. For this reason mid-region assays have been advocated as the preferred method of diagnosing hyperparathyroidism (HPT). Regardless of the assay system, all reported methods have presented clinical results supporting to a greater or lesser extent the usefulness of that particular assay.

Although the number of reports of new assays increases steadily, only 4 reports have appeared concerning the comparisons of different methods. The 1978 report of the European PTH Study Group (13) compared the ability of 12 laboratories to distinguish between sera of various clinical categories using their own assay systems, and concluded "provision of a PTH value without an accompanying description of the characteristics of the

assay system is worthless and uninterpretable". The large differences between results from separate laboratories was again evident in a 1979 comparison of 4 independent laboratories (14), a 1982 comparison of 5 commercial kits (15), and "in-house" results published in the 1984 European Study Group report (16). However, in this last study, performance by the same laboratories was much more uniform when using a kit of reagents distributed for the purposes of the study, demonstrating, not surprisingly, that the major source of variation was in the reagents themselves.

We here report the results of an assessment of some currently available commercial antisera (AS) and the clinical usefulness of several assay systems based on commercially available reagents, including 2 PTH kits.

MATERIALS AND METHODS

Reagents

Iodination buffer: 0.05 mol/L phosphate buffer:- ($NaH_2PO_4.2H_2O$ 1.528g/L, Na_2HPO_4 5.706g/L, in glass distilled water, adjusted to pH 7.4).

RIA buffer: as above plus NaCl 7.013g/L, Na_2EDTA 0.387g/L and thiomersal 0.1g/L.

Assay buffer: RIA buffer plus 1% human serum albumin (HSA) (Commonwealth Serum Laboratories, Melbourne, Aust.) and 300 units of aprotinin (Bayer, Trasylol) /mL.

Standards

Synthetic hPTH fragments 44-68, 44-68 (43Tyr), 53-84, and 53-84 (52Tyr) were obtained from Bachem Fine Chemicals, Torrance, CA. bPTH for iodination was obtained from Inolex Inc., Kankakee, IL. (lot 1515B001). bPTH for standards (MRC 71/324) was obtained from the National Institute for Biological Standards and Control, London, England. hPTH 1-84 was a generous gift from Dr. H. Keutmann, Endocrine Unit, Massachusetts General Hospital, Boston, MA.

Antisera

Wellcome Diagnostics, Beckenham, Kent, England were the source of AS BW 211/32, 266/5, 211/41, 802/7, 262, 1022/1, 1127/17 and 1127/23. Other AS were obtained from Calbiochem, San Diego, CA. (cat. no. 512525), Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA. (cat. no. CNR 242), Immunonuclear Corp., Stillwater, MN. (INC-MM. cat. no. 5102) and Institut National des Radioelements, Fleuris, Belgium (IRE AS-17).

Second Antibodies

Anti-chicken precipitating serum was obtained from Cambridge Nuclear (CNC 289N) and anti-guinea pig precipitating serum from Wellcome Diagnostics (RD 18). Anti-rabbit gamma-globulin AS for use as second antibody was prepared in sheep in response to purified rabbit gamma globulin antigen.

PTH kits

These were obtained from Wellcome Diagnostics (Dac-cel PTH), and Immunonuclear (human PTH MM RIA cat. no. 5100).

Pooled human hypoparathyroid serum was obtained from 3-4 confirmed hypoparathyroid patients receiving high dose vitamin D therapeutically and was stored in aliquots at -20°C until use.

Procedures

Affinity Constants

Affinity constants for AS were determined after either 3 or 5 day equilibrium incubations of labelled fragment (either hPTH 44-68 or 53-84 tyrosine analogs) with increasing amounts of the equivalent cold PTH fragment, and results calculated by Scatchard analysis.

Radio-iodination

bPTH, hPTH 44-68 (43Tyr) and hPTH 53-84 (52Tyr) were iodinated by the lactoperoxidase method (17). The peptide (3-8μg) was dissolved in 10μl of 0.001mol/L HCl, and mixed with 3μg of lactoperoxidase (Sigma Chemicals L-8503) dissolved in 10μL of iodination buffer and mixed. 10μL (1 mCi) of carrier free I^{125} (Amersham IMS.30) and 4μL of a solution of 10μL of 10vol hydrogen peroxide in 10 mL of deionised water were added, mixed, and the reaction stopped after 20-30 sec by the addition of

a mixture of 100μL each of potassium iodide (20mg/mL in RIA buffer) and 10% bovine serum albumin (Sigma A-8022) in RIA buffer containing 0.1% sodium azide. The mixture was transferred rapidly to an appropriate Sephadex column (G-75 for bPTH, G-50 for fragments), and eluted with RIA buffer containing 1% bovine serum albumin. After the void volume had been collected, 500μL fractions were collected and aliquots counted for radioactivity (LKB-Wallac Ultrogamma 1280). The peak fraction was retained and purified (immediately before adding label to an assay) by adsorption to microfine silica (QUSO) and elution with assay buffer adjusted to pH3. Label was used for up to 6 weeks after iodination. hPTH 53-84 does not bind to QUSO and was used without further purification.

Assay Conditions

These are summarised in Table 1. All "in-house" assays basically followed the method of Bouillon (18). Standards were serially diluted in assay buffer adjusted to pH 3 and assayed in triplicate (50μL/tube). 50μL of the pH 3 buffer was added to all other tubes. 100μL of patient serum or serum equivalent (see Table 1) was added followed by AS in 200μL of assay buffer and the tubes mixed. At the end of the first incubation period, approximately 10,000 cpm of labelled hormone or fragment in 100μL of assay buffer was added to each tube and mixed. The appropriate second antibody plus carrier serum was added in

100μL of buffer at the end of the second incubation and left overnight.

Separation was effected by the addition of 2mL of RIA buffer containing 1% Triton X-100 (Ajax Chemicals 1552) and centrifugation at 300xg for 20 min (Kontron TG6-A). Supernatant was decanted and the Triton X wash repeated. All incubations and centrifugation were carried out at 4°C.

After decanting, the precipitate was counted for radioactivity and results calculated by multiple iterative line fitting procedure, modified from the method of Burger (19). Assay sensitivity is defined as the lowest concentration at which the calculated PTH value is twice the standard deviation at that concentration ie SD(X)/X=0.5. Both kits were used as per the manufacturers' instructions. Values were calculated by the same method as for the "in-house" assays. Assay specificity was determined from standard curves of fragments, intact bPTH, intact hPTH or serially diluted pooled serum from 4 1° HPT patients.

Other Biochemical Measurements

Total calcium (Ca_T), albumin, creatinine, phosphate and chloride were all determined by standard biochemical methods. Ionized calcium (Ca^{++}) was determined on a Nova 2 ionized calcium analyser (Nova Biomedical, Newton, MA.).

TABLE 1

Assay Characteristics and Performance

	Assay Number					
	#1	#2	#3	#4	#5	#6
Antiserum	IRE	INC-MM	BW 1022/1	BW 1022/1	INC kit	Dac-cel Kit
Final dil^{n}	1/140000	1/75000	1/130000	1/130000	NA	1/150000
Standard	h44-68	h44-68	bPTH	h53-84	h44-68(43Tyr)	bPTH
Assay range[a]	5-500	5-500	10-250	10-500	31-1000	26-840
Label	h44-68 (43Tyr)		bPTH	h53-84 (52Tyr)	bPTH37-84	bPTH
Spec. activ. (uCi/ug)	100-150	100-150	40-50	100-150	NA	150[b]
Time course (hrs)	96:72	24:24	120:72	72:72	.25:2	4:16
Sep. method	2ndAB	2ndAB	2ndAB	2ndAB	2ndAB	2ndAB
Final vol. (uL)	450	450	450	450	500	300
Std. curve protein	Hypopara		6%HSA	6%HSA	none	hum.serum
B_o(%)	20-35	18-20	19-27	24-27	39	33

50% displac[a].	275	110	130	120	105	130
NSB (%)	0.2	0.3	1.5	0.3	0.7	5.4
Sensitivity[a]	5–10	5–10	10–15	10–15	6	14
Interassay CV(%)	16	15	18	14	9[b]	9[b]
Normal range[a]	<40	<20	<35	<20	10–55	<40
% normals detected	67	44	64	9	100	14
Recovery of standard (%)	80–110	68–104	NA	NA	80–110[b]	98–114[b]
Specificity[c] h44-68	1	1	NR	NR	NA	NR
h53-84	2	NR	1.2	1	NA	0.8[b]
h1-84	2.2	3	3	4.6	0.95[b]	1.5[b]
b1-84	0.7	NA	1	2.7	0.8[b]	1[b]

a pmol/L equivalents of assay standard
b manufacturers reported values
c ratio of added hormone to assay standard to produce 50% displacement of label
NA not available or determined
NR not reached (>500 pmol/L)
NSB non specific binding

Patients and Controls

Control sera for normal ranges were obtained from volunteer donors attending the Red Cross blood transfusion centre, Perth, W. Aust., and were not used if either Ca_T or Ca^{++} values were outside the normal range. At least 40 and up to 150 controls were used in the different PTH assay systems. Sera from the following patient groups were used:

Group 1 (proven 1° HPT)

42 1° HPT patients (80 samples) confirmed histologically after parathyroidectomy. 33 had adenomas, and 9 had hyperplasia. All had plasma creatinine <150μmol/L (normal 50-120μmol/L).

Group 2

11 biochemically 1° HPT patients (17 samples). These patients were accepted after clinical examination and/or review of case notes if they satisfied the following criteria:

1. At least 3 samples for Ca_T and albumin available.
2. Mean corrected Ca >2.65mmol/L (normal Ca_T 2.25-2.65 mmol/L, correction - for every albumin unit < or > 40g/L add or substract 0.02 mmol/L Ca) (20).
3. 1 plasma phosphate <0.85mmol/L (normal 0.8-1.4mmol/L).
4. 1 chloride value >102.5mmol/L (normal 98-106mmol/L) (21). Omitted if currently or recently on diuretic.
5. Plasma creatinine within 1 week of PTH sample <150μmol/L (normal 50-120μmol/L).

Group 3 (CRF)

25 chronic renal failure patients.

Group 4

11 patients with hypercalcaemia of malignancy, with or without metastases, concurrent HPT not excluded, creatinine <150μmol/L.

Group 5 (non-malignant hypercalcaemia)

9 patients (11 samples) with hypercalcaemia of varying etiology: sarcoidosis, vitamin D toxicity, hyperthyroidism, or milk alkali syndrome.

Because of limitations in sample numbers for the kit assays, not all patients were determined by kit. However, all group 1 patients and 6 from group 4 were measured in both kits and all "in-house" assays for direct comparison.

RESULTS

Initial evaluation of the 12 AS was done by determining their K_A for the two hPTH fragments 44-68 and 53-84 (Table 2). The Cambridge Nuclear (CNC) and Calbiochem AS exhibited low affinity for hPTH fragments. Preliminary assays with CNC AS confirmed that it was unsuitable for measurement of hPTH in serum. Using bPTH as label and standard good standard curves were obtained, but no serum values exceeded 25pmol equiv./L. Calbiochem AS and BW 802/7 were not examined further due to their low K_A values. BW 262 has a low titre and is not recommended by the manufacturers for human assay using bovine assay

TABLE 2

Affinity Constants of Antisera with PTH Fragments

	K_A ($\times 10^9$) L/mol		
Antiserum	hPTH 44-68	hPTH 53-84	Used in assay no.
Cambridge Nuc.	4.2	3.4	
Calbiochem	3.2	0.9	
BW 802/7	0.09	1.6	
BW 262	4.5	12.5	
BW 211/32	8.0	1.4	
BW 211/41	1.04	22.0	
BW 266/5	-	9.4	
BW 1127/17	-	8.3	
BW 1127/3	-	12.8	
BW 1022/1	3.2	25.6	#3,#4
IRE	24.8	17.5	#1
INC-MM	24.0	0.54	#2,#5

systems. K_A values for hPTH fragments were low and it was not investigated further. Like many others we had previously used BW 211/32 for some years until supply ceased and it was excluded from this comparative study. BW AS 266/5, 1127/17 and 1127/23 were received towards the end of the study and for that reason have not as yet been fully evaluated.

Although BW 1022/1 and 211/41 have similar K_A values for both PTH fragments tested, BW 211/41 was not found to be useful for assaying hPTH for other than chronic renal failure patients and was excluded from further comparisons. Trial assays suggested that BW 1022/1 was a suitable C-terminal AS and it was incorporated into two assay systems (Table 1, number 3 and number 4) using either bPTH or hPTH 53-84 as standard, affording some comparison between heterologous and "homologous" assays. The IRE and INC-MM AS were selected for mid-region assays using hPTH 44-68 as standard (Table 1, number 1 and number 2), and, as the INC-MM is nominally the same AS as used in the INC kit, this AS provided another comparison of the effects on AS performance of changing other assay components. General assay characteristics and performance are listed in Table 1.

The primary purpose of this paper is to compare the performance of these various assay systems in the discrimination of 1° HPT values from normal. For this purpose, two criteria were used:

Criterion 1

The generally used criterion that values greater than the upper limit of the normal (ULN) range are elevated.

Criterion 2

This attempts to make allowance for assay variability. For PTH values less than 50pmol/L the standard deviation (SD) due to the curve fitting procedure is 4-6pmol/L for all assays, and hence

(i) values more than 10pmol/L below the ULN are considered as normal.

(ii) values more than 10pmol/L above the ULN are clearly elevated, and all intermediate values are considered as equivocal.

DISCUSSION

With the exception of the IRE AS, all AS examined showed either a clear preference for one or other of the hPTH fragments tested. Several were eliminated from further consideration because of an inability to cross react favourably with hPTH. Of the 7 assay systems investigated in depth, the general characteristics (Table 1) were similar. The kits (numbers 5-6) and the INC "in-house" assay (number 2) were significantly faster, and the mid-region assays demonstrated increased sensitivity (number 1, number 2 and number 6). Assay system number 1 had a flatter standard curve than the remainder but this appeared to have no adverse effect on performance, and parallelism with

serially diluted pooled HPT serum was good. Normal ranges were approximately equivalent, but there was considerable difference in the percent of normal sera having detectable PTH (9-100%). Only the IRE AS (number 1) was able to detect both hPTH 44-68 and hPTH53-84 fragments, while relative affinity for intact hPTH varied from near equivalence for the INC kit to almost one fifth for the "in-house" h53-84 assay (number 4).

The ability of the assays to discriminate 1° HPT patients from normals varied widely (Table 3), and with no apparent correspondence with the components of the assay system. The reason for poor performance in individual assay systems was not simply that values were equivocal instead of clearly elevated, but that in the poor performance assays values were lower for all 1° HPT samples and more appeared in the "normal" area ie "false negatives" (Table 3, criterion 2(i)). The best assay of the 6 here investigated was a mid-region assay (number 1) and it has been suggested (7,8,9) that mid-region assays are to be preferred over C-terminal systems. Number 2 and number 5 are also mid-region assays but their performance was far less impressive than number 1 and did not outperform C-terminal assays numbers 3 and 6. Thus mid-region specificity *per se* is not a guarantee of improved performance. A similar argument indicates that the use of PTH fragments (either bovine or human) also does not guarantee a better assay. Thus the use of hPTH 53-84 with AS BW 1022/1 resulted in a relatively poor assay as

compared with the same AS using bPTH. Although this fragment has been used elsewhere (4) its successful use is apparently AS dependent.

Table 3 also shows the discriminatory ability of each assay for 1° HPT patients subdivided on the basis of average corrected total Ca. It is immediately apparent that regardless of the assay system used, discrimination decreases dramatically in patients with milder hypercalcaemia, who in general have milder or earlier HPT and are correspondingly more difficult to diagnose on other grounds. "Percent discrimination" is a widely used evaluation criterion for PTH RIA. However, our results indicate that such a criterion is useless without detailed biochemical data on the patient groups involved. Similarly, assay systems cannot be usefully compared unless the same group of patients is measured in each assay.

Because of the inability of PTH assays to completely and reliably discriminate 1° HPT patients from normal, clinical diagnosis invariably considers the PTH value in relation to concurrent Ca values for the patient. Thus it has become common practice for reports of new PTH assays to include a plot of iPTH versus either total or ionized Ca as an indication of the diagnostic value of the assay. Although this approach may be valid as a diagnostic indicator, it has no place in the comparison of assay systems when proven 1° HPT samples are used. For example, such a procedure for the assays described in this paper results

TABLE 3

Assay Performance - Discrimination of Proven 1° HPT Patients from Normal

	Assay No.					
	#1	#2	#3	#4	#5	#6
Antiserum Standard	IRE h44-68	INC-MM	1022/1 bPTH	h53-84	INC-MM 44-68(43tyr)	Daccel(BW) bPTH
Criterion 1 >(ULN)	86[a] (42)	69 (42)	79 (42)	58 (38)	48 (31)	74 (31)
Criterion 2(ii) >(ULN+10pmol/L) All patients	81 (42)	60 (42)	64 (42)	45 (38)	39 (31)	61 (31)
Patients with [b]Ca >3mmol/L Ca 2.7-2.99 Ca <2.7mmol/L	100 (9) 84 (19) 62 (13)	100 (9) 53 (19) 38 (13)	89 (9) 63 (19) 46 (13)	88 (8) 31 (16) 31 (13)	75 (8) 21 (14) 25 (8)	88 (8) 57 (14) 38 (8)
Criterion 2(i) <(ULN-10pmol/L) "false negatives"	9 (42)	21 (42)	19 (42)	31 (38)	42 (31)	16 (31)

a results are given as percentages, followed in parentheses by total number of patients in each category

b average (n>3) corrected total Ca

in % discrimination values ranging from 92% for assay number 4 to 97-98% for the other 5 assays, suggesting that most are of equal performance, which is clearly not the case. The improved results are obviously due largely to the discriminatory value of the Ca levels themselves.

Table 4 presents assay performance for 4 other patient categories. All assays have little problem discriminating the highly elevated PTH values characteristic of chronic renal failure patients, nor do they show elevated levels for non-malignant hypercalcaemia samples. Discrimination using "in-house" assays for biochemical HPT patients was slightly lower but followed the same pattern as for proven 1° HPT samples.

Hypercalcaemia of malignancy is a perennial problem area for PTH RIA, and most PTH assays produce significant numbers of either elevated or "inappropriate" levels of PTH in malignant patients. The question as to whether this finding reflects a high co-incidence of HPT with malignancy or else some assay interference has not been satisfactorily resolved. The subject has been discussed elsewhere (22) where it was also suggested that assays with greater discrimination of HPT from normal may also produce more "inappropriate" PTH levels in malignant hypercalcaemia. This suggestion is strongly supported by our results; there is a definite relationship between the percentage of 1° HPT samples discriminated from normal and the number of elevated PTH results from the malignant hypercalcaemia group.

TABLE 4

Assay Performance - % Elevated Values[a] for Various Patient Groups

Group Designation	n	Assay No. #1	#2	#3	#4	#5	#6
2 biochem HPT	(11)	64	55	64	64	-	-
3 CRF	(25)	100	100	96	100	-	100 (8)[b]
4 malig. Ca↑	(11)	33	8	17	17	0 (5)	29 (6)
5 nonmalig. Ca↑	(9)	0	0	0	0	-	-

a greater than 10pmol/L above ULN (criterion 2(ii))

b for assays #5 and 6, number of patients in parentheses

By the judicious combination of AS, label and standard it is possible to produce acceptable PTH assays for routine use, using commercially available materials. However, there do not appear to be any reliable guidelines as to the best mix of ingredients, and the ultimate performance of any assay system still relies on a trial and error approach.

ACKNOWLEDGEMENTS

The authors wish to thank Burroughs-Wellcome for the provision of PTH assay kits gratis for evaluation, and for the generous gift of samples of antisera 1127/17, 1127/23 and 266/5. Thanks are also due to Immunonuclear Corporation for PTH kits at half price for evaluation, and to the Red Cross Blood Transfusion Service, Perth, Western Australia for the provision of serum samples for normal ranges. Address requests for reprints to Dr. G K Worth, Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia, 6009.

REFERENCES

1. Martin KJ, Hruska K, Freitag J, Bellorin-Font E, Klahr S and Slatopolsky E. Clinical utility of radioimmunoassays for parathyroid hormone. Mineral Electrolyte Metab. 3: 283-290 (1980).

2. Broadus AE and Rasmussen H. Clinical evaluation of parathyroid function. Amer.J.Med. 70: 475-478 (1981)

3. Slatopolsky E, Martin K, Morrissey J and Hruska K. Current concepts of the metabolism and radioimmunoassay of parathyroid hormone. J.Lab.Clin.Med. 99, 309-316 (1982).

4. Hitzler, Schmidt-Gayk H, Spiropoulos P, Raue F and Hüfner M. Homologous radioimmunoassay for human parathyrin (residues 53-84). Clin.Chem. 28: 1749-1753 (1982).

5. Mallette LE. A radioimmunoassay for human parathyroid hormone utilising a goat anti-bovine PTH serum. Acta Endocrinol. 96: 215-221 (1981).

6. Atkinson MJ, Niepel B, Jüppner H et al. Homologous radioimmunoassay for human mid-regional parathyroid hormone. J.Endocrinol.Invest. 4: 363-366 (1981).

7. Marx SJ, Sharp ME, Krudy A, Rosenblatt M and Mallette LE. Radioimmunoassay for the middle region of human parathyroid hormone: studies with a radioiodinated synthetic peptide. J.Clin.Endocrinol.Metab. 53: 76-84 (1981).

8. Roos BA, Lindall AW, Aron DC et al. Detection and characterisation of small midregion parathyroid hormone fragment(s) in normal and hyperparathyroid glands and sera by immunoextraction and region specific radioimmunoassay. J.Clin.Endocrinol.Metab. 53: 709-721 (1981).

9. Jüppner H, Rosenblatt, Segre GV and Hesch R-D. Discrimination between intact and mid-C-regional PTH using selective radioimmunoassay systems. Acta Endocrinol. 102: 543-548 (1983).

10. Wood WG, Marschner I and Scriba PC. Tests on three antisera and subsequent development of radioimmunoassays for different regions of human parathyrin. Horm.Metab.Res. 11: 309-317 (1979).

11. Juan D. Differential diagnosis of hypercalcaemia. Postgrad.Med. 66: 73-81 (1979).

12. Mohr H, Hesch R-D, McIntosh CHS and Jüppner H. Parathyroid hormone fragments in secretory granules of human parathyroid adenomata. Europ.J.Clin.Invest. 10: 317-323 (1980).

13. European PTH Study Group. Interlaboratory comparison of radioimmunological parathyroid hormone determination. Europ.J.Clin.Invest. 8: 149-154 (1978).

14. Raisz LG, Yajnik CH, Bockman RS and Bower BF. Comparison of commercially available parathyroid hormone immunoassays in the differential diagnosis of hypercalcaemia due to primary hyperparathyroidism or malignancy. Ann.Intern.Med. 91: 739-740 (1979).

15. Gorog RH, Hakim MK, Thompson NW, Rigg GA and McCann DS. Radioimmunoassay of serum parathyrin: comparison of five commercial kits. Clin.Chem. 28, 87-91 (1982).

16. Minne HW. Quality control in parathyroid hormone radioimmunoassay: a multicentre study performed by the European Parathyroid Hormone Study Group. European J.Clin.Invest. 14: 16-23 (1984).

17. Thorell JI and Johansson BG. Enzymatic iodination of polypeptides with ^{125}I to high specific actvitiy. Biochim. Biophys.Acta 251, 363-369 (1971).

18. Bouillon R and de Moor P. Pathophysiological data obtained with a radioimmunoassay for human parathyroid hormone. Annales d'Endocrinol. 34: 657-668 (1973).

19. Burger HG, Lee VWK and Rennie GC. A generalised computer program for the treatment of data from competitive protein-binding assays including radioimmunoassay. J.Lab.Clin.Med. 80: 302-312 (1972).

20. Anonymous. Correcting the calcium. Brit.Med.J. 1: 598 (1977)

21. Wills MR and McGowan GK. Plasma chloride levels in hyperparathyroidism and other hypercalcaemic states. Brit.Med.J. 1: 1153-1156 (1964).

22. Boyd JC, Lewis JW, Slatopolsky E and Ladenson JH. Parathyrin measured concurrently with free or total calcium in the differential diagnosis of hypercalcaemia. Clin.Chem. 27: 574-579 (1981).