

Lack of Comparability of Intact Parathyroid Hormone Measurements among Commercial Assays for End-Stage Renal Disease Patients: Implication for Treatment Decisions

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Background: Variability among assays used to measure intact parathyroid hormone (iPTH) is of particular concern because of the routine use of iPTH assay results to guide management of osteodystrophy and calcium metabolism in patients with end-stage renal disease (ESRD). The aim of this study was to determine the extent to which results from commercially available iPTH assays diverge from results obtained with the Nichols Allegro® Intact PTH IRMA assay, which was used as evidence in the development of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) Clinical Practice Guidelines.

Methods: We divided EDTA plasma from 46 dialysis patients with ESRD and measured iPTH values with the following commercially available iPTH assays: Nichols' Allegro iPTH IRMA, Nichols Advantage® iPTH immunochemiluminescent assay (ICMA), Scantibodies' Total Intact PTH™ IRMA, DiaSorin's N-tact® iPTH IRMA, DPC's Coat-A-Count® iPTH IRMA, Roche's Elecsys® iPTH ICMA, and DSL's Active® iPTH IRMA.

Results: Method comparison showed considerable interassay differences in the measurement of iPTH in ESRD patients. iPTH values assessed by other methods ranged, on average, from 60% to 152% of the Nichols Allegro IRMA values. Of the 6 iPTH assays tested, only the Scantibodies Total Intact PT IRMA ($P = 0.7554$) and the Roche Elecsys iPTH ICMA ($P = 0.1327$) resulted in iPTH values not statistically different from those obtained with the Nichols Allegro iPTH IRMA.

Conclusions: Noncomparability among iPTH assays remains a distinct problem for the management of ESRD patients. These results should be taken into consideration when determining the course of medical treatment based on measured iPTH concentrations

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In 2003, the National Kidney Foundation published the Kidney Disease Outcomes Quality Initiative (K/DOQI),⁴ Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease (1). These guidelines contain specifications for both diagnosis and treatment of end-stage renal disease (ESRD) based upon intact parathyroid hormone (iPTH) values obtained by the Nichols Institute Diagnostics' Allegro® iPTH immunoradiometric assay (IRMA). However, most laboratories in the United States no longer use the Nichols Allegro iPTH IRMA to test ESRD patient specimens. Importantly, substantial variation has been detected between iPTH values measured using the Nichols Allegro iPTH IRMA and the more commonly used Nichols Advantage™ iPTH assay (reviewed in Ref (2)). Significant differences in observed iPTH values could alter the course of treatment for ESRD patients in a potentially harmful manner. Therefore, it is essential to determine the extent to which iPTH values obtained with other commercially available assays vary from those obtained using the K/DOQI referenced Nichols Allegro iPTH IRMA.

Materials and Methods

SAMPLES

EDTA plasma was collected from 46 patients with ESRD (stage 5 chronic kidney disease) at commercial clinical

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⁴ Nonstandard abbreviations: K/DOQI, Kidney Disease Outcomes Quality Initiative; ESRD, end-stage renal disease; iPTH, intact PTH; IRMA, immunoradiometric assay; ICMA, immunochemiluminescent assay; cat., catalog.

laboratories during the year 2003. These patients received hemodialysis 3 times weekly, and specimens were collected immediately before a hemodialysis session. Blood was collected in EDTA lavender-topped tubes, inverted gently 5 times, and then centrifuged at 3000 rpm for 10–15-min. Plasma was aliquoted into 7 cryovials (one for each iPTH assay) and stored at -20°C until the time of assay. All samples were processed within 1 h of blood collection. The persons executing the tests were all qualified according to the standards required for generating commercial clinical laboratory results.

INTACT PTH ASSAYS

Intact PTH concentrations were measured according to the manufacturer's exact instructions using the following commercially available assays: Nichols' Allegro IRMA (Cat. 40–2170), Nichols' Advantage ICMA (Cat. 62–7022), Scantibodies' Total Intact™ IRMA (Cat. 3KG600), DiaSorin's N-tact® PTH SP IRMA (Cat. 26100), DPC's Coat-a-Count® IRMA (Cat. IKPH1), Roche's Elecsys® ICMA (Cat. 11972103), DSL's Active® IRMA (Cat. DLS-8000). This study did not seek to reconfirm the test reproducibility as stated in the directional inserts of the commercial clinical assays. A reference standard was not required nor used.

STATISTICAL ANALYSIS

Data were analyzed with Microsoft Excel 2003 and Analyze-it software (Ver. 1.73). The linear regression was performed with the Y intercept set to zero. The Wilcoxon signed-ranks test (with a confidence interval of 95%) was used to determine if iPTH values obtained with each assay were substantially different from those obtained with the Nichols Allegro IRMA.

Results and Discussion

iPTH serum concentrations were measured in 46 patients with ESRD using 7 different commercially available iPTH assays. The results of this assay comparison are summarized in Table 1. The percentage difference between each tested assay and the Nichols Allegro IRMA was calculated for each patient sample ($n = 46$), and the mean

percentage differences are shown in a bar chart format (Fig. 1). For purposes of comparison, the Nichols Allegro IRMA is treated as the reference assay and presumed to be 100% accurate in terms of PTH recovery. The percentage of PTH recovered was calculated as (test assay iPTH value)/(Nichols Allegro IRMA iPTH value) for the other iPTH assays evaluated in this study and presented in Table 1. While the Scantibodies' Total Intact PTH IRMA, DPC iPTH IRMA, and Roche's iPTH ICMA assays exhibited only small differences ($< \sim 10\%$), the other three assays, Nichols' Advantage IRMA, DiaSorin's IRMA, and DSL's IRMA, exhibited considerable differences ($\geq \sim 40\%$) compared with the Nichols' Allegro IRMA iPTH. When iPTH values measured using each assay were plotted against the values measured using the Nichols' Allegro IRMA, linear regression analysis performed with Microsoft Excel 2003 revealed that only the Scantibodies' Total Intact IRMA and the Roche Elecsys ICMA data sets possessed slopes approximately equivalent (~ 0.05) to 1 (Table 1). All data sets possessed r values >0.946 , indicating linear correlation with the Nichols Allegro IRMA assay. The Wilcoxon signed-ranks test for matched pairs was used to determine whether a substantial difference existed between the Nichols Allegro IRMA data set and each of the other iPTH assays. $P >0.05$ indicates a substantial difference with respect to values obtained using the Nichols Allegro IRMA. No substantial differences were observed between the Nichols Allegro IRMA iPTH data and the Scantibodies Total Intact PTH IRMA ($P = 0.7554$) and Roche iPTH ICMA ($P = 0.1327$). The Nichols Advantage ICMA ($P <0.0001$), DiaSorin's N-tact IRMA ($P = 0.0001$), DPC's Coat-a-Count IRMA ($P <0.0006$), and DSL's Active IRMA ($P = 0.0001$) data sets were all substantially different ($P <0.01$) from the Nichols Allegro IRMA data set. While the percentage recoveries were found in general to be close to the slopes generated from linear regression, however, in some cases, such as the Nichols Advantage PTH, the slope of the linear regression was not in very good agreement with the mean percentage recoveries. We note the limitations of linear regression in that the relationships between these assays across the

Table 1. Comparison of commercially available iPTH assays with the Nichols' Allegro IRMA.

	Nichols Allegro IRMA	Nichols Advantage ICMA	Scantibodies Total Intact IRMA	DiaSorin N-tact IRMA	DPC Coat-a-Count IRMA	Roche Elecsys ICMA	DSL Active IRMA
Mean iPTH, pg/mL	226	294	227	122	196	218	328
Standard deviation, pg/mL	184	221	193	95	154	169	280
Average differences, pg/mL ^a	0	67	1	-104	-30	-8	102
Standard deviation, pg/mL	0	66	31	99	53	29	109
Normal range, pg/mL	10–65	10–65	14–66	13–54	12–72	15–65	9–55
Average percentage recoveries ^b	100%	152%	99%	60%	93%	103%	147%
Standard deviation	$\sim 0\%$	$\sim 117\%$	$\sim 19\%$	$\sim 16\%$	$\sim 23\%$	$\sim 21\%$	$\sim 29\%$
Slopes ^c	1.000	1.241	1.015	0.521	0.843	0.941	1.460

^a Average differences = test assay iPTH value–Nichols Allegro iPTH value; $n = 46$.

^b Average percentage recovery = (test assay iPTH value)/(Nichols Allegro iPTH value).

^c Linear regression analysis for data presented in Figure 2.

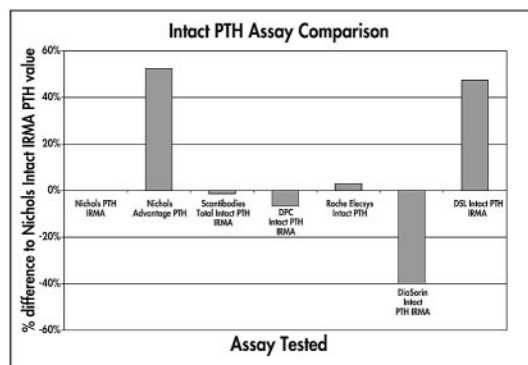


Fig. 1. Intact PTH assay comparison of commercial iPTH assays vs Nichols' Allegro IRMA iPTH.

The percentage difference between each tested assay and the Nichols Allegro IRMA was calculated for each patient sample ($n = 46$), and the mean percentage differences are plotted.

dynamic measurement range and the Nichols Allegro IRMA assay cannot be assumed to be linear. Therefore, the authors consider the difference in PTH recoveries to be a more reliable indicator of assay differences. It is also noted that the normal ranges for all of these assays is essentially the same (Table 1). The fact that the differences in recoveries among these assays are not indicated by differences in their respective normal ranges suggests that factors such as PTH fragment interferences or nonlinearities for increased samples might account for these assay differences. It must also be noted that it cannot be assumed that assays do not change (2). Additionally, the difference in the amount of hPTH (1–84) calibrators present in the assays is probably one of the major factors that contribute to the differences in the percentage recoveries and slopes. D'Amour et al. (3) reported that when the differences in the hPTH (1–84) calibrators were corrected, the iPTH values generated with the different assays became similar. Therefore, application of a common standard may partially address the differences among those assays used in this study (2). However, other factors, such as antibody purification, assay matrix and differences in the assay formats could also contribute to the differences in recoveries (Fig 2).

The National Kidney Foundation (K/DOQI) Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease indicate that vitamin D sterols should be given when serum concentrations of iPTH reach ≥ 300 pg/mL (1). To determine how the described interassay differences might affect administration of vitamin D sterols to ESRD patients, we determined the number of patient samples with iPTH concentrations ≥ 300 pg/mL for each assay (Table 2). The Nichols Allegro IRMA, on which these K/DOQI guidelines are based, produced 13 patient samples with iPTH concentrations above the cutoff. Of the other 6 assays tested, only the Scantibodies assay produced the same 13 patient samples, where the Roche and DPC assays produced 12 and 11 patient samples, respectively. Strikingly, the DiaSorin

assay produced only 3 patient samples with iPTH concentrations above the cutoff, while the Nichols Advantage ICMA and DSL assays produced 17 and 18 patient samples, respectively.

The K/DOQI guidelines further recommend the dosage of vitamin D sterols to be administered. For example, patients with 300–599 pg/mL iPTH would receive 0.5–1.5 μ g of oral calcitriol, those with 600–999 pg/mL iPTH would receive 1–4 μ g, and those with ≥ 1000 pg/mL iPTH would receive 3–7 μ g. To determine how interassay differences might affect the dosing of vitamin D sterols, we divided the patients with iPTH concentrations ≥ 300 pg/mL into the 3 treatment groups (Table 2). On the basis of the Nichols Allegro IRMA results, only 2 patients would receive the intermediate dose of calcitriol and no patients would receive the high dose. In contrast, the DSL Active IRMA results indicated that 6 patients would receive the intermediate dose and 1 patient would receive the high dose.

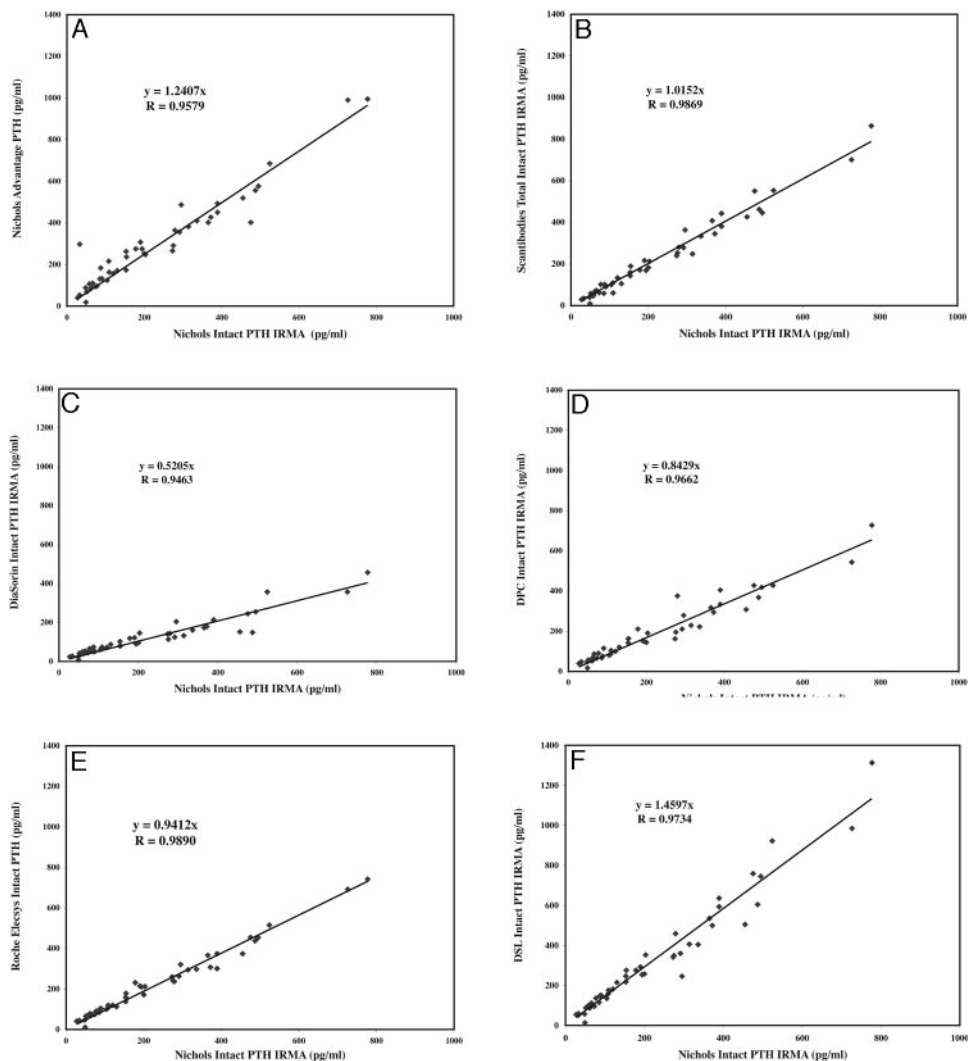
The National Kidney Foundation K/DOQI Clinical Practice Guidelines include suggestions for the treatment of ESRD based upon serum iPTH concentrations. However, these guidelines were based on an assay that is no longer commonly used in clinical settings. The results of this study suggest that many of the iPTH assays used instead of the Nichols Allegro IRMA do not give comparable results. Because iPTH values are commonly used to determine whether an ESRD patient would benefit from a parathyroidectomy, these results indicate that interassay differences could contribute to the under or over-prescription of parathyroidectomies. The assay with a substantial overestimation in iPTH (Nichols' Advantage iPTH) was the most widely used iPTH assay for ESRD testing in the US (2). There was an unexplained increase in the number of parathyroidectomies in the United States between 1999 and 2002 (4). Additionally, overdosing of vitamin D can have serious medical consequences, including calcification (5) and dynamic bone disease. One explanation for the increase in parathyroidectomies is that, in 1999, there was an upward shift in the most widely used iPTH assay for ESRD patients (the Nichols Advantage iPTH assay) (2).

There are several possible explanations for the described lack of comparability among assays. First, the iPTH assays described in this study rely on 2 different antibodies to detect intact PTH, 1 specific for a broad N-terminus region, and 1 specific for a broad C-Terminus region of the PTH molecule. However, each company generally develops its own specific antibodies and measurement methods; therefore, differences in antibody specificity and affinity for PTH as well as differences in the methods of measurement could partially account for the observed lack of comparability. This is especially important to consider for ESRD patients, as their serum often accumulates high concentrations of large and small PTH fragments as a result of decreased kidney function (6–7). These PTH fragments may interfere with the ability

Fig. 2. Linear regression analysis of commercial iPTH assays vs Nichols Allegro IRMA.

iPTH values for 46 patient samples were measured with the indicated commercial iPTH assays (y-axis) and plotted against iPTH values obtained with the Nichols Allegro IRMA (x-axis). Linear regression analysis was performed with the intercept set to 0, and the curves are shown:

- 2A, Nichols Advantage PTH ICMA vs Nichols Allegro IRMA;
 2B, Scantibodies Total Intact PTH IRMA vs Nichols Allegro IRMA;
 2C, DiaSorin Intact PTH IRMA vs Nichols Allegro IRMA;
 2D, DPC Intact PTH IRMA vs Nichols Allegro IRMA;
 2E, Roche Elecsys Intact PTH vs Nichols Allegro IRMA;
 2F, DSL Intact PTH IRMA vs Nichols Allegro IRMA.



of the two antibodies to bind exclusively to their intended target (8–10) of intact PTH.

Second, the standards or calibrators used to determine iPTH concentrations can vary from company to company. While many companies use synthetic human iPTH as the source of the standard, these may be suspended in different matrices which would have the potential to cause assay interference (11, 12).

Finally, the calibrators typically consist of purified synthetic intact PTH. Therefore, their composition is not

reflective of the ESRD patient's specimen, which contains many other serum proteins in addition to PTH. This noncommutability between calibrator and patient specimen may further contribute to the observed interassay differences.

In conclusion, the results described herein suggest that the lack of comparability among iPTH assays remains a distinct problem in the diagnosis and treatment of secondary hyperparathyroidism in patients with ESRD. The development of a universal reference panel for use in the

Table 2. Interassay differences relating to K/DOQI recommended iPTH levels for Vitamin D sterol administration.

	Nichols Allegro IRMA	Nichols Advantage ICMA	Scantibodies Total Intact IRMA	DiaSorin N-tact IRMA	DPC Coat-a-Count IRMA	Roche Elecsys ICMA	DSL Active IRMA
iPTH >300, pg/mL ^a	13	17	13	3	11	12	18
300–599 pg/mL ^b	11	14	11	3	10	10	11
600–999 pg/mL	2	3	2		1	2	6
>1000 pg/mL							1

^a Number of patient samples (n = 46) with iPTH values >300 pg/mL.

^b Number of patient samples with the indicated iPTH serum concentrations.

standardization of commercial iPTH assays could help resolve this problem.

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