8-28-2018

Trueness, precision and stability of the LIAISON 1-84 parathyroid hormone (PTH) third-generation assay: comparison to existing intact PTH assays

Andre Valcour

Claudia Zierold

Frank A. Blocki

Douglas M. Hawkins

Kevin J. Martin

See next page for additional authors

Recommended Citation

Valcour A, Zierold C, Blocki FA, Hawkins DM, Martin KJ, Rao SD, and Bonelli F. Trueness, precision and stability of the LIAISON 1-84 parathyroid hormone (PTH) third-generation assay: comparison to existing intact PTH assays. Clin Chem Lab Med 2018; 56(9):1476-1482.

Andre Valcour*, Claudia Zierold, Frank A. Blocki, Douglas M. Hawkins, Kevin J. Martin, Sudhaker D. Rao and Fabrizio Bonelli

Trueness, precision and stability of the LIAISON 1-84 parathyroid hormone (PTH) third-generation assay: comparison to existing intact PTH assays

https://doi.org/10.1515/cclm-2018-0217
Received February 27, 2018; accepted March 7, 2018; previously published online May 11, 2018

Abstract

Background: Over the past few decades, parathyroid hormone (PTH) immunoassays have progressed through successive generations resulting in increased specificity and accuracy for detecting circulating PTH. With the introduction of third-generation assays, in which the biologically active PTH(1-84) is specifically targeted, the PTH(7-84) and other fragments are not detected. The specific recognition of only PTH(1-84) whole molecule allows for more reliable standardization and calibration than with the existing assays.

Methods: Samples from patients on hemodialysis or with primary hyperparathyroidism and apparently healthy subjects were examined in different collection matrices (EDTA plasma, unspun EDTA plasma and SST) stored for 0, 24 or 72 h at room temperature to reflect the prevailing sample collection methods, shipping and processing conditions of centralized labs in the United States. Samples were analyzed by the LIAISON 1-84 PTH and N-TACT assays, and by three additional commercially available intact PTH assays.

Results: Defined samples, prepared using two different standards (WHO 95/646 international standard and the synthetic Bachem PTH(1-84)), show little bias with the LIAISON 1-84 PTH assay, but not with the other intact PTH assays. Furthermore, PTH is stable for up to 72 h in plasma, but less stable in serum beyond 24 h.

Conclusions: The FDA-approved LIAISON 1-84 PTH assay is accurate and reliably measures the biologically active PTH molecule in plasma or serum stored at room temperature for up 72 and 24 h, respectively.

Keywords: accuracy; immunoassay; parathyroid hormone; stability; standards; trueness.

Introduction

Parathyroid hormone (PTH) is essential for regulation of calcium and phosphate homeostasis [1]. Intraoperative assessment of parathyroidectomy discloses a detectable half-life of approximately 3 min [2]. Accurate measurement of serum PTH levels is critical for the diagnosis of hyper- and hypoparathyroid disorders and for monitoring patients with chronic kidney disease.

Although PTH(1-84) is the biologically active hormone, PTH carboxyl fragments that lack the N-terminal amino acid sequence required for biological activity (i.e. PTH(7-84) and others) are also present in the circulation as a result of glandular secretion or peripheral degradation of the hormone [3].

Over the past 2–3 decades PTH immunoassays have progressed through a succession of generations resulting in increased specificity and trueness for detecting PTH. The commonly used intact PTH (iPTH) assays (second generation) not only measure the biologically active PTH(1-84) but also detect, to varying degrees, inactive fragments such as the PTH(7-84) fragments, and the measurements are thus widely variable [4, 5]. With the introduction of third-generation assays in which only the biologically active PTH(1-84) whole peptide is specifically targeted, greater specificity is realized enabling proper standardization, greater trueness and consistent measurements. Unlike third-generation PTH assays, intact PTH assays are not commutable due to their variable reactivity to inactive PTH(7-84) fragments [6].

Against this historic composite, PTH stability studies to date have been performed with various outcomes

***Corresponding author: Andre Valcour,** PhD, LabCorp, Center for Esoteric Testing, 1447 York Court, Burlington, NC 27215, USA, Phone: +(336) 436-3854, Fax: +(336) 436-0618, E-mail: ValcouA@labcorp.com
Claudia Zierold, Frank A. Blocki and Fabrizio Bonelli: DiaSorin Inc., Stillwater, MN, USA
Douglas M. Hawkins: Scottsdale Scientific LLC, Scottsdale, AZ, USA
Kevin J. Martin: Division of Nephrology, Saint Louis University, St. Louis, MO, USA
Sudhaker D. Rao: Division of Endocrinology, Diabetes, Bone and Mineral Metabolism, Henry Ford Health System, Detroit, MI, USA

attributable to assay variability, temperature and matrix parameters [7]. The need for a multifactorial study was confirmed in a recent study by Schleck et al. [8] whose study design was similar to ours. However, the present study design is more expansive using more patient types, more platforms and most importantly, longer storage times that best represent the current routine flow within the centralized U.S. laboratory system. Furthermore, traceability to the World Health Organization (WHO) International Standard was explored to determine trueness of the methods.

Materials and methods

The experimental design for the study is shown in Figure 1: hemodialysis, primary hyperparathyroid or apparently healthy subjects (n = 5 each) were enrolled from three different sites: Saint Louis University, Henry Ford Hospital and LabCorp. For each patient, nine tubes (three time points for each of three sample types) were collected: (1) K_2 EDTA plastic lavender top vacutainers and centrifuged within 30 min of draw (plasma), (2) K_2 EDTA plastic lavender top vacutainers stored uncentrifuged (unspun plasma) and (3) serum separator gold top vacutainers centrifuged within 45–60 min to separate serum from the clot (SST). Tubes were stored at room temperature for 0, 24 or 72 h, followed by storage in 1 mL aliquots at −80 °C. Unspun tubes were centrifuged after storage immediately prior to aliquoting. Following collection, the frozen samples were shipped to LabCorp for PTH measurement on five different analyzers whose assay parameters are described in Table 1. The data were analyzed by a multifactor analysis of variance fitted separately for each tube type, with storage duration and patient type as factors. Analytical variability, taken from the manufacturers' reported precision studies, was comparable between assays with repeatability parameters ranging from ~3 to 5 %CV or less, with the 1-84 PTH LIAISON assay having imprecisions of 3.1%–4.9% and 4.0%–7.2% for repeatability and reproducibility, respectively (Table 1) [9].

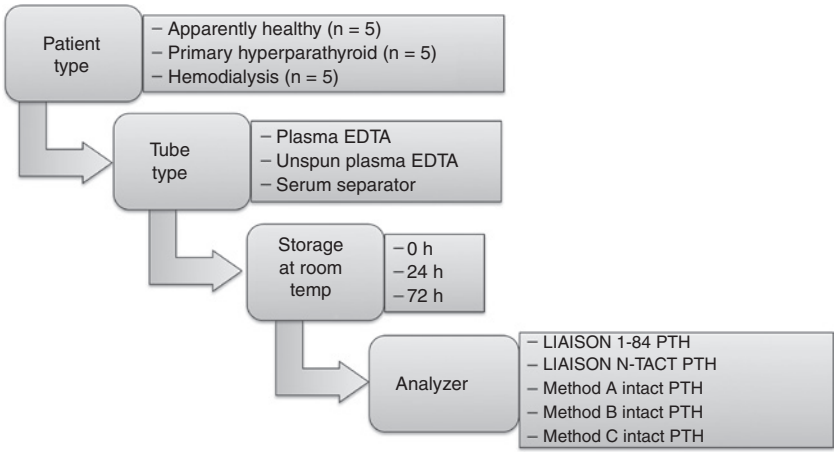

Figure 1: Experimental design.
Patient types, matrices, storage times and analyzer platforms are outlined culminating in a total of 675 measurements.

Table 1: Description of parameters of the assays used.

	LIAISON 1-84 PTH	LIAISON N-TACT II	Method A[a]	Method B[a]	Method C[a]
Assay measuring range	4.0–1800 pg/mL	3.0–1900 pg/mL	2.5–1900 pg/mL	1.20–5000 pg/mL	3.0–2500 pg/mL
Calibration standard	Bachem synthetic 1-84 peptide traceable to WHO 95/646	WHO 95/646 standard traceable to WHO 95/646	Purified human 1-84 PTH traceable to WHO 79/500	Not reported	Not reported
Epitope specificity					
Capture	39–84 fragment	39–84	39–84	1–37	44–84
Detection	Serine 1	1–34	1–34	38–84	1–34
Detection chemistry	ABEI[b]	ABEI	Acridinium ester	Ruthenium	Alkaline phosphatase
Precision (%CV)					
Within-run	3.1%–4.9%	3.9%–6.1%	3.4%–5.2%	0.6%–2.8%	4.2%–5.7%
Total	4.0%–7.2%	5.1%–8.9%	4.6%–7.8%	1.6%–3.4%	6.3%–8.8%

[a]Data were taken from manufacturer's kit instructions for use. [b]ABEI, N-(4-aminobutyl)-N-ethyl-isoluminol.

For trueness assessment, the WHO International Standard PTH(1-84), human, recombinant NIBSC code: 95/646 (Hertfordshire, UK) [10, 11], or Bachem synthetic PTH(1-84) (PN H-1370, Torrance, CA, USA) was spiked into charcoal-stripped, heat-inactivated human serum at concentrations ranging from 0 to 1000 pg/mL. The samples were analyzed for PTH content by the different methods. The distribution of inactive PTH fragment was assessed in 1533 hemodialysis patients by measuring both PTH(1-84) with the LIAISON method, and iPTH with Method A because of its high cross-reactivity with inactive fragments [4]. The fragment content was calculated as (iPTH-PTH(1-84))/iPTH and expressed as % of total iPTH.

The study protocol was approved by the respective Institutional Review Boards of the respective sites.

Results

One of the major criticisms of commercial PTH assays has been that none of the intact PTH assays have been calibrated to the 95/646 WHO PTH1-84 standard [12]. This is largely due to the detection of PTH fragments in the iPTH assays. The data in Figure 2 show WHO standards prepared at concentrations ranging between 0 and 1000 pg/mL and the corresponding measured values of PTH derived from the different methods. The LIAISON 1-84 PTH measures the WHO recombinant PTH(1-84) standard with little bias as evidenced by its close alignment with the line of identity, thereby corroborating the reliable standardization, calibration and commutability of this FDA-approved assay. Closely aligned data were observed when trueness was assessed using the synthetic Bachem PTH(1-84) standard (data not shown).

All samples in the stability study were normalized to the time = 0 h plasma sample for analysis. In Figure 3, average deviations for all 15 patients from the baseline plasma sample at each time point are plotted for the five different platforms and three sample types. Box-Cox methodology recommended that the analysis be made on log-transformed values, which are shown on the y-axes of these plots. Table 2 presents the same data expressed as % change from the plasma sample (T = 0 h). Little variability was observed for plasma and

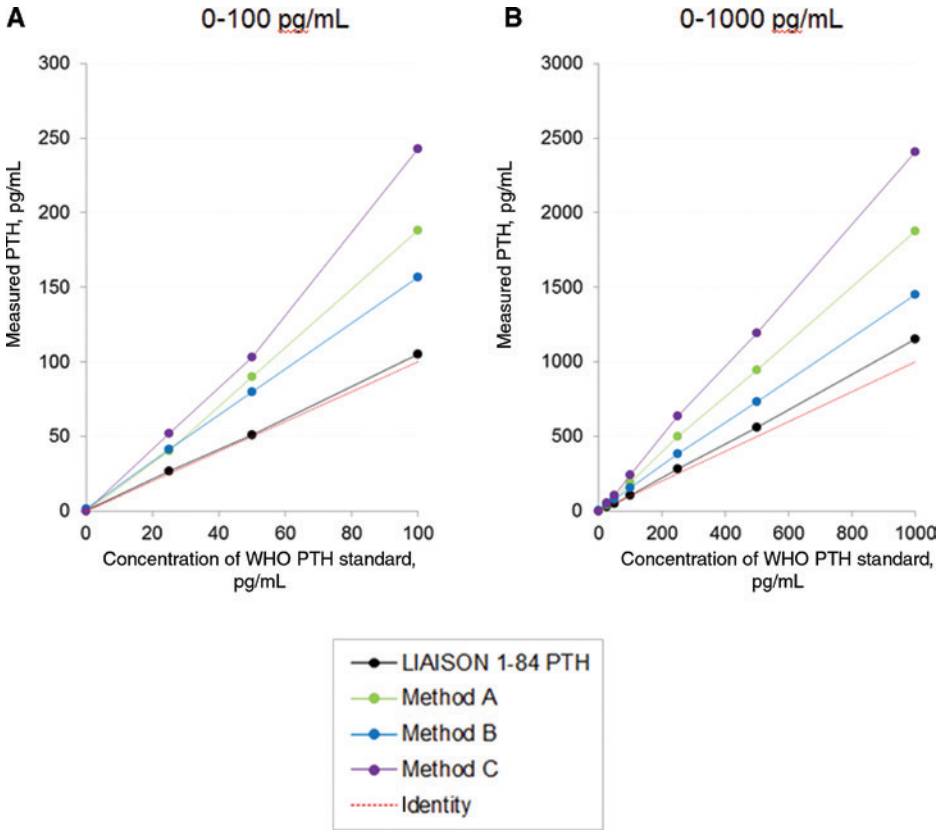

Figure 2: WHO standards prepared at concentrations ranging between 0 and 1000 pg/mL in charcoal-stripped heat-inactivated human serum were assessed with each assay and plotted against each standard.
(A) Alignment at PTH(1-84) concentrations between 0 and 100 pg/mL (range for apparently healthy and most primary hyperparathyroid).
(B) Alignment over the entire tested range (0–1000 pg/mL).

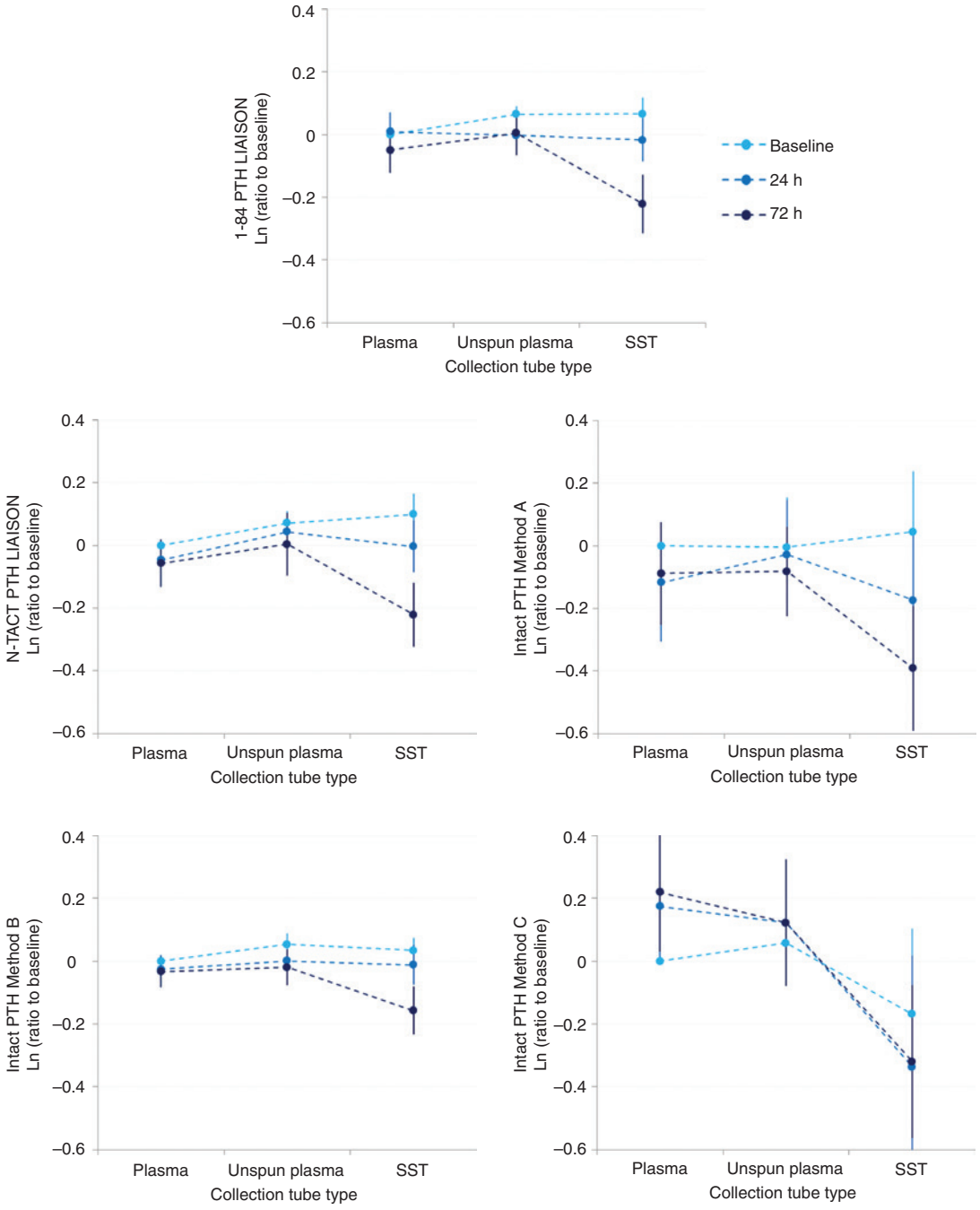

Figure 3: Deviation from reference sample.
The log-transformed values of the ratio of measurement to the reference value (plasma at T = 0 h) were plotted as an average with 95% confidence interval for the 15 subjects in the study for each sample type and storage time. The corresponding values expressed in percent change are shown in Table 2.

unspun plasma over the first 24-h storage period for the PTH(1-84) and iPTH on the LIAISON analyzers and for iPTH by Method B. Method A and Method C were more variable but not significantly different from baseline for plasma and unspun plasma. Most notable, however, is that serum is significantly less stable than plasma when samples are stored at room temperature for 72 h regardless of platform, even when separated from the clot by centrifugation within 1 h. The stability of PTH in unspun plasma for 72 h at room temperature was confirmed in an independent study with hemodialysis patients, where the levels of PTH(1-84) were 99% ± 5% from the

Table 2: Percent change from the baseline plasma sample corresponding to the log transformed data in Figure 3.

Tube type	Storage at RT	LIAISON 1-84 PTH		LIAISON N-TACT PTH		Method A intact PTH		Method B intact PTH		Method C intact PTH	
		Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Plasma	0 h	100%		100%		100%		100%		100%	
	24 h	102%	95%–108%	96%	91%–101%	93%	80%–106%	98%	93%–103%	125%	104%–145%
	72 h	96%	88%–104%	95%	88%–103%	95%	84%–106%	97%	92%–102%	133%	104%–162%
Unspun plasma	0 h	107%	104%–110%	108%	103%–112%	103%	91%–115%	106%	102%–110%	109%	97%–121%
	24 h	101%	94%–107%	105%	99%–112%	101%	88%–114%	100%	96%–105%	116%	103%–129%
	72 h	101%	94%–109%	102%	92%–112%	95%	84%–105%	99%	93%–105%	122%	95%–149%
SST	0 h	107%	101%–114%	111%	103%–120%	110%	94%–127%	104%	99%–108%	92%	77%–107%
	24 h	99%	91%–107%	101%	92%–110%	89%	75%–103%	99%	92%–107%	82%	66%–98%
	72 h	82%	73%–89%	82%	72%–91%	72%	59%–84%	86%	79%–93%	78%	65%–91%

Table 3: Analysis of variance of the changes in PTH from baseline plasma samples.

	0 h				24 h				72 h			
	Effect	SE	t	p-Value	Effect	SE	t	p-Value	Effect	SE	t	p-Value
LIAISON 1-84 PTH												
Plasma	Ref	Ref			0.065	0.033	1.95		0.066	0.033	1.99	<0.05
Plasma unspun	0.010	0.033	0.29		−0.002	0.033	−0.07		−0.017	0.033	−0.52	
SST	−0.050	0.033	−1.49		0.005	0.033	0.14		−0.221	0.033	−6.62	<0.001
LIAISON N-TACT												
Plasma	Ref	Ref			0.071	0.038	1.89		0.099	0.038	2.62	<0.01
Plasma unspun	−0.046	0.038	−1.21		0.044	0.038	1.16		−0.003	0.038	−0.08	
SST	−0.057	0.038	−1.50		0.003	0.038	0.09		−0.221	0.038	−5.85	<0.001
Method B iPTH												
Plasma	Ref	Ref			0.053	0.026	2.06	<0.05	0.034	0.026	1.30	
Plasma unspun	−0.016	0.026	−0.61		0.000	0.026	0.01		−0.023	0.027	−0.86	
SST	−0.033	0.026	−1.29		−0.020	0.026	−0.77		−0.161	0.026	−6.07	<0.001
Method A iPTH												
Plasma	Ref	Ref			−0.005	0.061	−0.08		0.044	0.061	0.72	
Plasma unspun	−0.101	0.063	−1.62		−0.029	0.061	−0.47		−0.177	0.063	−2.83	<0.01
SST	−0.089	0.061	−1.44		−0.082	0.061	−1.33		−0.394	0.063	−6.30	<0.001
Method C iPTH												
Plasma	Ref	Ref			0.058	0.100	0.57		−0.169	0.100	−1.68	
Plasma unspun	0.174	0.100	1.74		0.121	0.100	1.21		−0.337	0.100	−3.36	<0.01
SST	0.219	0.100	2.18	<0.05	0.122	0.100	1.22		−0.320	0.100	−3.18	<0.01

T = 0 sample and levels of iPTH were 97% ± 6% (data not shown).

Multifactor analysis of variance (Table 3) indicates statistical significance at the 5%, 1% and 0.1% levels. Although some of the marginally statistically significant differences may be explained as Type I errors from multiple testing, the larger significant effects observed across the board for SST samples stored at room temperature for 72 h likely represent true instability. Method A and Method C show the largest effects, which are likely a combination of both sample instability and assay imprecision.

Discussion

In the present study, we found that PTH is stable for up to 72 h in EDTA plasma from hemodialysis, primary hyperparathyroid and apparently healthy subjects across a wide range of PTH levels. Blood collected in K_2 EDTA plastic lavender top vacutainers stored uncentrifuged at room temperature for up to 72 h, and then centrifuged, also demonstrated remarkable PTH stability.

Serum provides a convenient sample type for PTH testing because its collection obviates the need for collecting a second sample for other tests that are frequently

ordered concomitantly (calcium, vitamin D metabolites). However, PTH measurements from unfrozen serum samples are accurate only if testing is performed within 24 h of collection. Alternatively, serum can be frozen at collection for subsequent testing on site or at a centralized laboratory. The advantage of testing from a single matrix is countered by the extra effort required for transferring serum to a secondary container, freezing the samples and thawing/mixing the sample prior to testing. In recognition of this, laboratories might consider collecting an EDTA whole blood sample for transfer at the point of collection for local testing or transportation to a central laboratory for processing and testing.

Although results generated by three of the five methods evaluated were quite consistent, two methods exhibited greater variability as revealed by their much larger confidence intervals (Figure 3). Methods A and C have greater cross-reactivity to the PTH(7-84) fragments that are known to be present at higher levels in hemodialysis patients [13]. Because greater instability in hemodialysis vs. normal or PHPT samples was not observed (data not shown), the larger confidence intervals likely represent a greater inherent imprecision of these two assays that is well beyond their stated analytical variability.

Our statistical approach, multifactor analysis of variance, differs from the approach taken by Schleck et al. [8]. Significant observations in the multifactor setup (given by multiple patient types, multiple sample matrices, multiple storage durations and multiple assay platforms) are best identified using a multiway analysis, which better controls Type I error rates, and borrows strength by pooling information from different cells, which enables more power [14]. By contrast, doing a collection of paired tests between these different combinations of factors can lead to false positives (Type I error) due to the multiplicity of all the test statistics generated, and at the same time lose power resulting in a failure to identify factors that actually matter (Type II errors).

PTH assays are notably quite erratic and provide dissimilar values depending upon the manufacturer's utilization of components with widely variable fragment cross-reactivity and different standardization regimens [4]. In this study, average PTH values were 101, 274, 238, 189 and 189 pg/mL for the LIAISON 1-84 PTH, LIAISON N-TACT and Methods A, B and C, respectively. Although efforts by the PTH working group to standardize PTH assays are ongoing, transitioning to the third-generation PTH assays affords immediate opportunity for true and standardized measurements, as these PTH(1-84) specific assays can be aligned with WHO recombinant standards [11] as well as synthetic PTH(1-84). In hemodialysis patients, there is a wide distribution of percent PTH-fragment content (Figure 4). These representative data from 1533 hemodialysis samples illustrate that patients with similar levels of iPTH and quite different levels of active PTH(1-84) will respond differently to treatment, emphasizing the importance of measuring the active molecule with third-generation assays that are standardized and accurate.

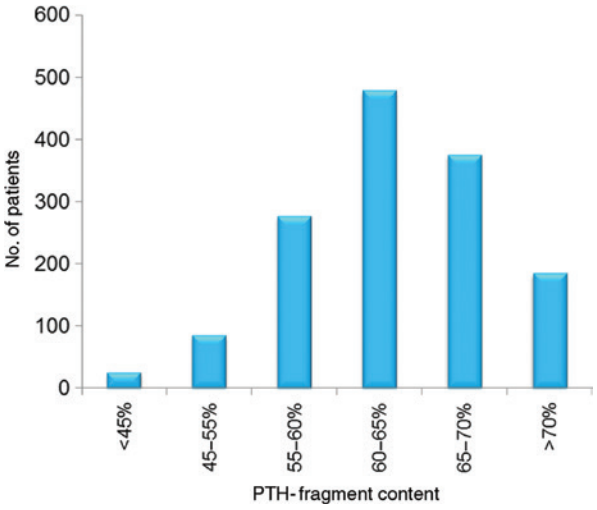

Figure 4: Frequency of fragment content in hemodialysis patients. Representative PTH fragment was assessed in 1533 hemodialysis samples by measuring both PTH(1-84) with the LIAISON method, and iPTH with Method A (high cross-reactivity with inactive fragments [4]). The fragment content was calculated as (iPTH-PTH(1-84))/iPTH and expressed as % of total iPTH.

Although the strength of our study resides in the statistical analysis and extended storage times, its major limitation was the inability to normalize to plasma samples collected and measured immediately without freezing [6]. The design of this study engaged multisite collection and centralized testing, which did not allow for such a control. Although Cavalier et al. showed average declines of ~16% and ~26% in iPTH assays following 1 and 3 months storage at −80 °C, respectively, in this study the samples were all processed similarly to mitigate or effectively normalize the effects of freezing.

Acknowledgments: We would like to thank Amy Mosman, Elizabeth Warner and Ray Ann Moreland, coordinators at Saint Louis University, Henry Ford Health System and LabCorp, respectively, for their hard work and valuable contribution.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This study was supported by DiaSorin Inc. (Stillwater, MN, USA) and LabCorp (Burlington, NC, USA).
Employment or leadership: AV is an employee of LabCorp, CZ, FAB, FB are employees of DiaSorin.
Honorarium: None declared.
Competing interests: The funding organization(s) played a role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Broadus AE. Mineral balance and homeostasis. In: Favus MJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. Philadelphia: Lippincott Williams & Wilkins, 1999:105–11.
2. Leiker AJ, Yen TW, Eastwood DC, Doffek KM, Szabo A, Evans DB, et al. Factors that influence parathyroid hormone half-life: determining if new intraoperative criteria are needed. JAMA Surg 2013;148:602–6.
3. Berne RM, Levy MN. Endocrine regulation of calcium and phosphate metabolism. In: Berne RM, Levy MN, editors. Physiology. St. Louis, MO: CV Mosby Company, 1983:954–60.
4. Souberbielle JC, Boutten A, Carlier MC, Chevenne D, Coumaros G, Lawson-Body E, et al. Inter-method variability in PTH measurement: implication for the care of CKD patients. Kidney Int 2006;70:345–50.
5. Sturgeon CM, Sprague S, Almond A, Cavalier E, Fraser WD, Algeciras-Schimnich A, et al. Perspective and priorities for improvement of parathyroid hormone (PTH) measurement – A view from the IFCC Working Group for PTH. Clin Chim Acta 2017;467:42–7.
6. Cavalier E, Plebani M, Delanaye P, Souberbielle JC. Considerations in parathyroid hormone testing. Clin Chem Lab Med 2015;53:1913–9.
7. Hanon EA, Sturgeon CM, Lamb EJ. Sampling and storage conditions influencing the measurement of parathyroid hormone in blood samples: a systematic review. Clin Chem Lab Med 2013;51:1925–41.
8. Schleck ML, Souberbielle JC, Delanaye P, Plebani M, Cavalier E. Parathormone stability in hemodialyzed patients and healthy subjects: comparison on non-centrifuged EDTA and serum samples with second- and third-generation assays. Clin Chem Lab Med 2017;55:1152–9.
9. DiaSorin. LIAISON® 1-84 PTH Assay, in Packaging Insert/Instructions for Use. US 49675, 2017-11.
10. Burns C, Moore M, Sturgeon C, Ellis A, Hockley J, Rigsby P. WHO International Collaborative Study of the proposed 1st International Standard for Parathyroid Hormone 1-84, human recombinant. Geneva, Switzerland: WHO Press, 2009:1–24.
11. WHO. International Standard Parathyroid Hormone 1-84, human, recombinant. NIBSC Code 95/646. Controls NIfBSa, Editor 2010, National Institute for Biological Standards and Controls. Version 9.0, dated 08/02/2010.
12. Seaborg E. Variability in parathyroid hormone assays: better standardization on the way? Kidney News 2017;9:1.
13. Chang JM, Lin SP, Kuo HT, Tsai JC, Tomino Y, Lai YH, et al. 7-84 parathyroid hormone fragments are proportionally increased with the severity of uremic hyperparathyroidism. Clin Nephrol 2005;63:351–5.
14. Box GE, Stuart JS, Hunter WG. Statistics for Experimenters, 2nd ed. Hoboken, NJ, USA: Wiley-Interscience, 2005.