# Unveiling a new era with liquid chromatography coupled with mass spectrometry to enhance parathyroid hormone measurement in patients with chronic kidney disease

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**Precise determination of circulating parathyroid hormone (PTH) concentration is crucial to diagnose and manage various disease conditions, including the chronic kidney disease–mineral and bone disorder. However, the lack of standardization in PTH assays is challenging for clinicians, potentially leading to medical errors because the different assays do not provide equivalent results and use different reference ranges. Here, we aimed to evaluate the impact of recalibrating PTH immunoassays by means of a recently developed LC-MS/MS method as the reference. Utilizing a large panel of pooled plasma samples with PTH concentrations determined by the LC-MS/MS method calibrated with the World Health Organization (WHO) 95/646 International Standard, five PTH immunoassays were recalibrated. The robustness of this standardization was evaluated over time using different sets of samples. The recalibration successfully reduced inter-assay variability with harmonization of PTH measurements across different assays. By recalibrating the assays based on the WHO 95/646 International Standard, we demonstrated the feasibility for standardizing PTH measurement results and adopting common reference ranges for PTH assays, facilitating a more consistent interpretation of PTH values. The recalibration process aligns PTH results obtained from various immunoassays with the LC-MS/MS method, providing more consistent and reliable measurements. Thus, establishing true standardization across all PTH assays is crucial to ensure consistent interpretation and clinical decision-making.**

*Kidney International* (2023) ■, ■–■; https://doi.org/10.1016/j.kint.2023.09.033

KEYWORDS: CKD-MBD; immunoassay; LC-MS/MS; parathormone; standardization; vitamin D

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**Received 5 July 2023; revised 19 September 2023; accepted 25 September 2023**



## Lay Summary

Accurate diagnosis and treatment of chronic kidney disease–related mineral and bone disorders hinge on measuring parathyroid hormone (PTH). Unfortunately, current PTH tests often yield inconsistent results, complicating clinical practice. To address this, standardizing PTH measurement methods is essential. Achieving this standardization requires advanced mass spectrometry techniques and understanding whether certain substances in the blood of chronic kidney disease and hemodialyzed patients affect PTH measurements. Recent advances in mass spectrometry revealed that a potentially problematic PTH fragment (7–84) was absent in patients' blood, alleviating concerns. In addition, oxidized PTH was not detected and circulating fragments did not interfere PTH assays. As a result, we explored recalibrating 5 different PTH kits on to a precise liquid chromatography tandem mass spectrometry reference method. The outcomes were promising, aligning PTH results across various immunoassays with the reference method. This calibration process promises more reliable and consistent PTH measurements, ultimately enhancing patient care by reducing result variability.

Beyond its paramount importance in diagnosing and managing various endocrine conditions such as primary and secondary hyperparathyroidism, hypoparathyroidism, and pseudohypoparathyroidism, the measurement of parathyroid hormone (PTH) is routinely conducted in patients with chronic kidney disease (CKD). The Kidney Disease: Improving Global Outcomes 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder

recommends monitoring PTH levels in CKD patients beginning in CKD G3a and suggests maintaining PTH levels of hemodialyzed (HD) patients at ∼2 to 9 times the upper limit of the normal (ULN) values of the assay used.[1] Yet, a recommendation that treatments should be based on multiples of a ULN value is quite unique and is a circumvolution because of the lack of standardization of PTH assays.[2] Such a lack of standardization is unfortunately a real source of confusion for clinicians in their daily practice, potentially leading to significant medical errors.[3,4]

Several reasons contributed so far to the current lack of standardization in PTH assays. The first one is the presence of circulating PTH fragments alongside the bioactive 1 to 84 PTH peptide.[5] These fragments, which are N-terminal or C-terminal truncated forms of PTH, circulate in the blood owing to liver metabolism of the active peptide or direct secretion by the parathyroid glands.[6–8] These fragments, which are eliminated by the kidney, have a longer half-life than 1 to 84 PTH itself,[9,10] accumulate in the blood of CKD patients,[11–13] and potentially interfere with second generation PTH assays (referred as "intact" PTH assay). Indeed, such assays are supposed to recognize, with various cross-reactivities, a family of large C-terminal fragments referred to as "non-(1 to 84)" PTH.[14] This is not the case of third generation immunoassays (also referred as "whole" or "bioactive" PTH assays) because such assays incorporate an anti–N-terminal antibody directed toward the first 4 amino acids of the peptide, eliminating the issue of cross-reactivity with PTH fragments.[15,16]

The second reason for the lack of standardization in PTH assays is calibration. Indeed, despite the availability of the 1 to 84, human, recombinant World Health Organization (WHO) International Standard (IS) NIBSC 95/646, differences in calibration remain, which can be due to noncommutability of the WHO material and/or its incorrect use by assay manufacturers. Consequently, the recovery of the same amount of the WHO 95/646 PTH IS can range from 100% to >250% depending on the assay used.[3]

Finally, the lack of a formally recognized reference measurement procedure, a gold standard method providing true values against which any commercial assay could be calibrated, further contributes to the lack of standardization in PTH assays.

In this study, we aimed at evaluating the impact of a recalibration of 5 PTH immunoassays, representing both second and third PTH generations assays, on the liquid chromatography tandem mass spectrometry (LC-MS/MS) method we recently developed. This method is indeed calibrated against the WHO 95/646 PTH IS and has the potential to become a reference measurement procedure.[17]

## METHODS

We used the second and third generation PTH assays from Roche on the cobas, the second and third generation assays from DiaSorin on the LIAISON analyzer, and the third generation PTH assay from Fujirebio on the LUMIPULSE instrument. The characteristics of these methods are detailed in Table 1 for reference. As our gold standard, we used the LC-MS/MS method that we recently developed.[17] Notably, this method distinguishes itself by eliminating the use of antibodies during the sample preparation, ensuring complete independence from any cross-reactivity concerns. It is calibrated against the WHO 95/646 IS, providing a robust reference framework. Finally, our LC-MS/MS method has undergone extensive validation for 1 to 84 PTH from 5.7 to 873 ng/l and exhibits a measurement uncertainty of <5.6%.

We prepared a calibration panel constituted of 40 pools of leftover ethylenediamine tetraacetic acid plasma samples by carefully mixing at least 10 different leftover samples indiscriminately originating from our daily routine together to constitute a single pool. The samples were selected on the basis of their assigned nominal value determined through our routine method (DiaSorin LIAISON third generation PTH), and pools were constituted to span the measuring range.

We then prepared 2 validation panels. For validation panel 1, we selected 138 leftover ethylenediamine tetraacetic acid plasma samples, 58 from CKD patients {19 from G4 (estimated glomerular filtration rate [GFR] between 15 and 29 ml/min per 1.73 $m^2$), 19 from G3b (estimated GFR between 30 and 44 ml/min per 1.73 $m^2$), and 20 from G3a (estimated GFR between 45 and 59 ml/min per 1.73 $m^2$) categories}, 37 from HD patients, and 43 from non-CKD patients (estimated GFR >60 ml/min per 1.73 $m^2$). For validation panel 2, we selected 109 other leftover ethylenediamine tetraacetic acid plasma samples: 52 from CKD patients (16 from G4, 17 from G3b, and 19 from G3a categories), 37 from HD patients, and 20 from non-CKD patients.

The ethylenediamine tetraacetic acid plasma samples used for panel preparation were stored at −20 °C for less than a month and had not been previously frozen. After preparation, panels were stored at −80 °C until measurement, which occurred within the same month. For each panel, a fresh WHO 95/646 ampoule was used to establish the calibration curve for the LC-MS/MS method. The measurements of the calibration, validation, and validation 2 panels were conducted at intervals of a minimum of 3 months, ensuring the use of different lots for immunoassays. All samples were measured in singlicates using both the 5 immunoassays and the LC-MS/MS method.

On the basis of the results of the calibration panel, we established regression equations of each of the immunoassays versus LC/MS-MS. We then used these equations to "recalibrate" the immunoassays on the LC-MS/MS method, and we verified the robustness of this calibration on the 2 validation panels. To evaluate the clinical impact of recalibration, we investigated the classification of non-CKD subjects and CKD patients using both the LC-MS/MS method and the different immunoassays on the basis of a standardized ULN value. Furthermore, we examined the classification of HD patients before and after recalibration by following the Kidney Disease: Improving Global Outcomes guidelines[18] and considering 2 to 9 times multiples of the standardized ULN value.

## RESULTS

Of the 40 constituted pools of the calibration panel, 1 was above the measuring range for the DiaSorin second generation assay (>1900 ng/l) and was thus discarded. The spanning range of the 39 remaining pools was 14 to 533, 16 to 1200, 16 to 436, 8 to 355, 6 to 315, and 5 to 251 ng/l for Roche second generation, DiaSorin second generation, Roche third





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**Table 1 | Characteristics of second and third generation PTH immunoassays as disclosed by the manufacturers**

| Method | Intra-assay CV (%) | Interassay CV (%) | Expected values (ng/l) | Generation | N-terminal antibody and target | C-terminal antibody and target | Traceability | Measuring range (ng/l) |
|---|---|---|---|---|---|---|---|---|
| Roche Cobas Elecsys PTH | <1.3 | <2 | 15–65 | Second | Mouse monoclonal 26–32 | Mouse monoclonal 37–42 | Commercially available RIA. Claims a mean recovery of 100% ± 4% of the WHO 95/646 IS | 1.2–5000 |
| Roche Cobas Elecsys PTH (1–84) | 0.9–7.6 | 1.6–11.4 | 15–57 | Third | Mouse monoclonal N-terminal part | Mouse monoclonal C-terminal part | WHO 95/646 IS | 5.5–2300 |
| DiaSorin LIAISON N-TACT PTH Gen II | 1.3–5.7 | 2.8–4.2 | 15–87 | Second | Goat polyclonal 1–34 | Goat polyclonal 39–84 | Internal preparation of human *recombinant* 1–84 PTH | 3–1900 |
| DiaSorin LIAISON 1–84 PTH | 3.0–5.9 | 5.4–9.0 | 7–39 | Third | Polyclonal N-terminal | Polyclonal C-terminal | Internal preparation of *synthetic* human 1–84 PTH | 4–1800 |
| Fujirebio LUMIPULSE G whole PTH | 1.1–4.1 | 1.4–4.1 | 5–36 | Third | Goat polyclonal | Goat polyclonal | WHO 95/646 IS | 4–5000 |

CV, coefficient of variation; IS, International Standard; PTH, parathyroid hormone; RIA, radioimmunoassay; WHO, World Health Organization.

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generation, DiaSorin third generation, Fujirebio third generation, and LC-MS/MS method, respectively.

The regression equations of these 39 pools measured with each immunoassay versus LC-MS/MS and the correlations coefficients are presented in Table 2 and Figure 1.

These equations were then applied to the PTH results of the samples of the 2 validation panels. The results obtained from the 2 sets of samples before and after recalibration are presented in Table 3. Before recalibration, the mean PTH concentrations were 138, 258, 104, 89, 77, and 66 ng/l for Roche second generation, Roche third generation, DiaSorin second generation, DiaSorin third generation, Fujirebio third generation, and LC-MS/MS method, respectively, in validation panel 1. After recalibration, the corresponding values were 63, 62, 57, 67, and 66 ng/l for the 5 immunoassays, respectively. Concerning the samples of validation panel 2, the corresponding mean concentrations before calibration were 82, 157, 288, 111, 95, and 86 ng/l for the same methods. After recalibration, the mean values for immunoassays were 71, 68, 60, 71, and 74 ng/l, respectively.

Before recalibration, the average bias between the immunoassays presenting the highest and lowest biases (i.e., Fujirebio third generation and DiaSorin second generation) compared to LC-MS/MS ranged from +9% to +224% in validation panel 1 and from +9% to +223% in validation panel 2. After recalibration, the average bias decreased to −0.8% and −6.8% in validation panel 1 and from −2.5% Q21 to −12.3% in validation panel 2 for these 2 immunoassays. The Roche third generation assay's bias against LC-MS/MS also decreased after recalibration (from +71% to −21.5% in validation panel 1 and from +57% to −24.9% in validation panel 2) but to a lesser extent compared to the other assays. The overall mean bias decreased from 86.9% to −7.2% in validation panel 1 and from 81.7% to −11.1% in validation panel 2 after recalibration.

To perform the clinical validation, it was necessary to establish a standardized ULN value. Because a ULN for the LC-MS/MS method had not been established yet, we decided to use the average of the 5 recalibrated ULN values provided for each kit, obtained through the regression equations. The manufacturers' original ULN values were 65, 87, 57, 39, and 36 ng/l for Roche second generation, DiaSorin second generation, Roche third generation, DiaSorin third generation, and Fujirebio third generation, respectively. After recalibration, these values were adjusted to 30, 26, 27, 31, and 31 ng/l, respectively. The average of these recalibrated ULN values was calculated to be 30 ng/l, which was consequently considered as the standardized ULN value to be applied as the reference value for both the LC-MS/MS method and the 5 recalibrated immunoassays. Accordingly, Figures 2 and 3 display the distribution of the results obtained in non-CKD subjects and CKD patients, respectively, before and after recalibration according to the ULN value and Figure 4 displays the results of HD patients according to 2 to 9 times the ULN value before and after recalibration. Regarding these 73 HD patients, using raw values of 60 and 270 ng/l (i.e., 2 × 30 and 9 × 30 ng/l) as targets, 1

**Table 2 | Regression equations and correlation coefficients of the calibration panel's 39 samples on the LC-MS/MS reference method** Q31

| Y | X | | | | |
|---|---|---|---|---|---|
| | Roche second generation | DiaSorin second generation | Roche third generation | DiaSorin third generation | Fujirebio third generation |
| LC/MS-MS = | $0.45X + 0.58$ | $0.21X + 7.21$ | $0.60X - 7.1$ | $0.73X - 1.8$ | $0.83X + 2.6$ |
| $r^2$ | 0.973 | 0.988 | 0.991 | 0.989 | 0.983 |

LC-MS/MS, liquid chromatography tandem mass spectrometry. Q32

patient was misclassified by the Roche second generation assay in the low range (value considered as >60 ng/l whereas it was lower with the LC-MS/MS method) and 5 were misclassified by the Roche third generation assay (values considered as <270 ng/l whereas they were higher). Figure 5 illustrates the representative outcome for a non-CKD subject, a CKD patient, and a HD patient before and after recalibration of the immunoassays using the LC-MS/MS method. Before recalibration, the non-CKD subject exhibited an LC-MS/MS concentration of 27 ng/l. This measurement initially translated to PTH concentrations spanning from 33 ng/l (using the Fujirebio third generation assay) to 77 ng/l (as determined by the DiaSorin second generation assay). However, after recalibration, the same patient's values were refined to a range falling between 22 ng/l (Roche third generation assay) and 30 ng/l (Fujirebio third generation assay). Likewise, in the case of a CKD patient with initial LC-MS/MS PTH concentrations of 81 ng/l and immunoassays PTH concentrations ranging from 99 ng/l (measured using the Fujirebio third generation assay) to 289 ng/l (measured using the DiaSorin second generation assay), the recalibration led to a refined range spanning from 67 ng/l (Roche third generation assay) to 85 ng/l (Fujirebio third generation assay) after recalibration. Finally, an HD patient presenting an LC-MS/MS PTH value of 142 ng/ml initially displayed a PTH concentration range of 181 to 609 ng/mL before recalibration. After recalibration, the PTH concentrations ranged from 117 to 153 ng/l.

## DISCUSSION

The key finding of this study suggests that it is now feasible to standardize all PTH assays, regardless of the assay methodology (second or third generation immunoassay). This breakthrough has the potential to bring significant improvements in the management of chronic kidney disease–mineral and bone disorder. In 2006, Souberbielle *et al.* highlighted the misleading nature of the 150 to 300 ng/l National Kidney Foundation Kidney Disease Outcomes Quality Initiative raw values used as targets for PTH in HD patients.[4,19] The authors highlighted that despite high correlation among the assays ($r$ values ranging from 0.975 to 0.994), the 15 assays tested (13 second generation and 2 third generation assays) could produce significantly divergent results. This discrepancy had the potential to lead to significant clinical errors, as individual patients might be classified as within, below, or above the Kidney Disease Outcomes Quality Initiative target range depending on the specific assay used. The alarming

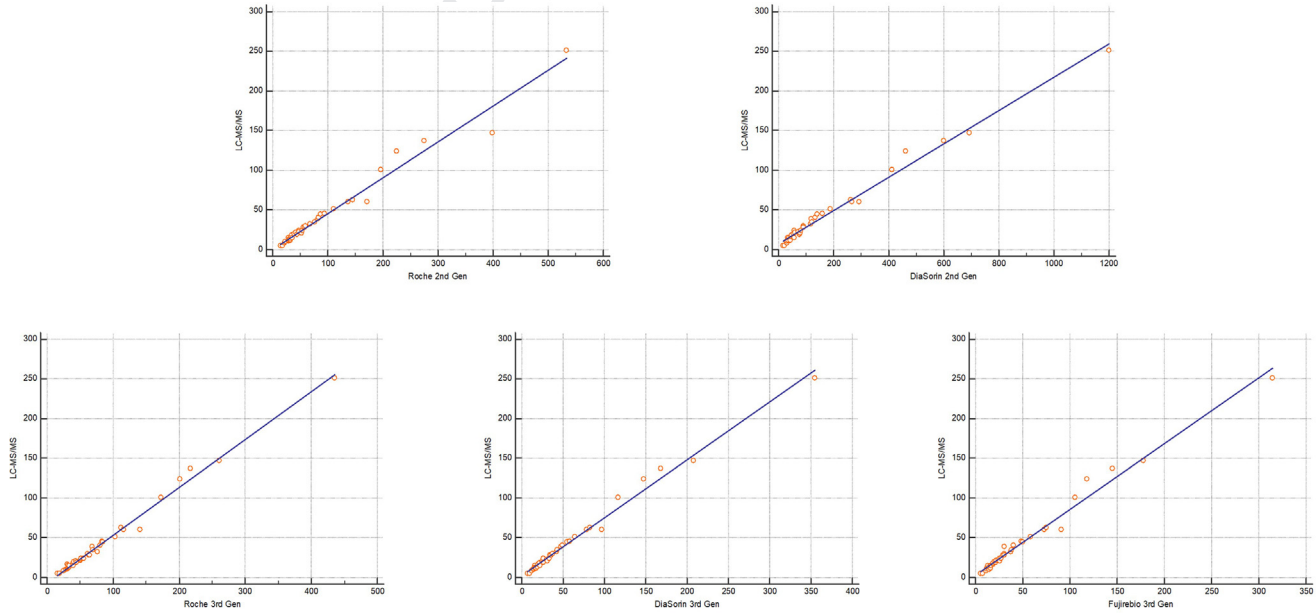


**Figure 1 | Scatter diagram depicting the regression line of the calibration panel's 39 samples for each immunoassay compared to the reference liquid chromatography tandem mass spectrometry (LC-MS/MS) method (measurement units are nanograms per liter).** 2nd Gen, second generation; 3rd Gen, third generation.





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**Table 3 | Characteristics of the 2 validation panels before and after recalibration of the samples on the reference LC-MS/MS method** Q33

| | Validation panel 1 | | | | | | Validation panel 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Before recalibration* | | | | | | | | | | | |
| XXXX | LC-MS/MS | Roche 2nd Gen | DiaSorin 2nd Gen | Roche 3rd Gen | DiaSorin 3rd Gen | Fujirebio 3rd Gen | LC-MS/MS | Roche 2nd Gen | DiaSorin 2nd Gen | Roche 3rd Gen | DiaSorin 3rd Gen | Fujirebio 3rd Gen |
| Mean (ng/l) | 65.7 | 138 | 257.5 | 104.2 | 89.3 | 77.0 | 81.5 | 156.8 | 287.5 | 111.1 | 95.2 | 85.8 |
| Mean bias (%) | | 104 | 224 | 71 | 27 | 9 | | 102 | 223 | 57 | 17.9 | 9.2 |
| Overall bias (%) | 86.9 | | | | | | 81.7 | | | | | |
| Overall CV (%) | 46.1 | | | | | | 59.0 | | | | | |
| | *After recalibration* | | | | | | | | | | | |
| | LC-MS/MS | Roche 2nd Gen | DiaSorin 2nd Gen | Roche 3rd Gen | DiaSorin 3rd Gen | Fujirebio 3rd Gen | LC-MS/MS | Roche 2nd Gen | DiaSorin 2nd Gen | Roche 3rd Gen | DiaSorin 3rd Gen | Fujirebio 3rd Gen |
| Mean (ng/l) | 65.7 | 62.9 | 61.5 | 55.6 | 67.1 | 66.4 | 81.5 | 71.4 | 67.8 | 59.8 | 71.4 | 73.7 |
| Mean bias (%) | | −5.5 | −6.8 | −21.5 | −1.2 | −0.8 | | −7.2 | −12.3 | −24.9 | −8.9 | −2.5 |
| Overall bias (%) | | | −7.2 | | | | | | −11.1 | | | |
| Overall CV (%) | | | 12.0 | | | | | | 20.5 | | | |

2nd Gen, second generation; 3rd Gen, third generation; CV, coefficient of variation; LC-MS/MS, liquid chromatography tandem mass spectrometry.

implication was that contradictory treatment approaches could have been recommended for a single HD patient on the basis of the choice of PTH assay.

To address this issue, the Kidney Disease: Improving Global Outcomes proposed using multiples of the ULN value established by assay manufacturers as PTH targets for HD patients. Undoubtedly, this approach significantly reduced the disparities in classifying HD patients based on PTH target ranges.[20] However, a potential weakness of this proposition regarding the complexity of establishing PTH reference values has been emphasized.[21] It is now recognized that factors such as vitamin D status, age, GFR, and potential ethnicity of the subjects included in the population used to establish PTH reference values have significant implications for determining

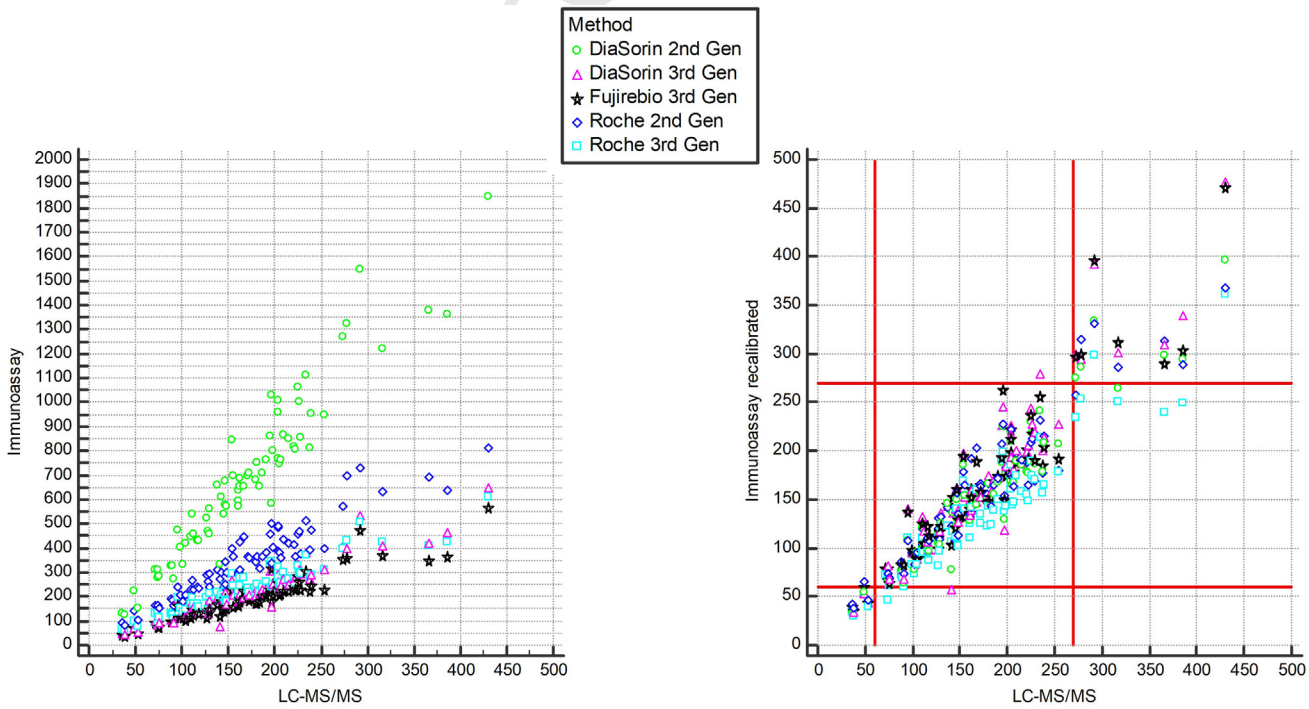


**Figure 2 | Comparison of the results obtained in non–chronic kidney disease subjects before (left) and after (right) recalibration.** The red solid bars correspond to 30 ng/l, which is the average of the 5 recalibrated upper limit of normal values provided for each kit, obtained through the regression equations (measurement units are nanograms per liter). 2nd Gen, second generation; 3rd Gen, third generation; LC-MS/MS, liquid chromatography tandem mass spectrometry.





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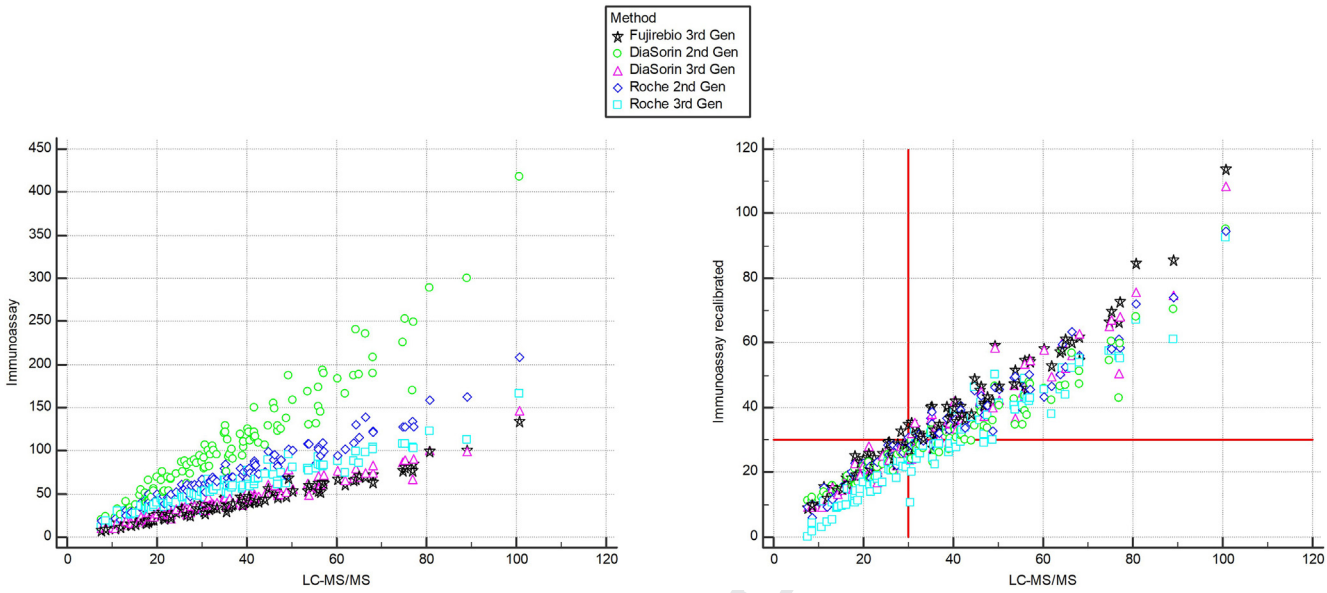


**Figure 3 | Comparison of the results obtained in chronic kidney disease patients before (left) and after (right) recalibration.** The red solid bars correspond to 30 ng/l, which is the average of the 5 recalibrated upper limit of normal values provided for each kit, obtained through the regression equations (measurement units are nanograms per liter). 2nd Gen, second generation; 3rd Gen, third generation; LC-MS/MS, liquid chromatography tandem mass spectrometry.

the PTH ULN value.[22–24] Many experts agree that the most effective solution to address this problem would be to achieve true standardization of all PTH assays, ensuring that regardless of assay methodology, all assays generate the same or reasonably similar results for a given sample. This approach would also facilitate the adoption of a unified ULN value, irrespective of the specific PTH assay used. Establishing such a singular ULN value would necessitate comprehensive analysis across diverse, multiethnic cohorts of healthy individuals spanning various ages, all devoid of primary hyperparathyroidism and any instigator of secondary hyperparathyroidism such as vitamin D deficiency, decreased GFR (among many others), and measurement of the samples with the candidate LC-MS/MS reference method.[22–24] Although the necessity for such a study is paramount and falls within the purview of international scientific organizations dedicated to bone

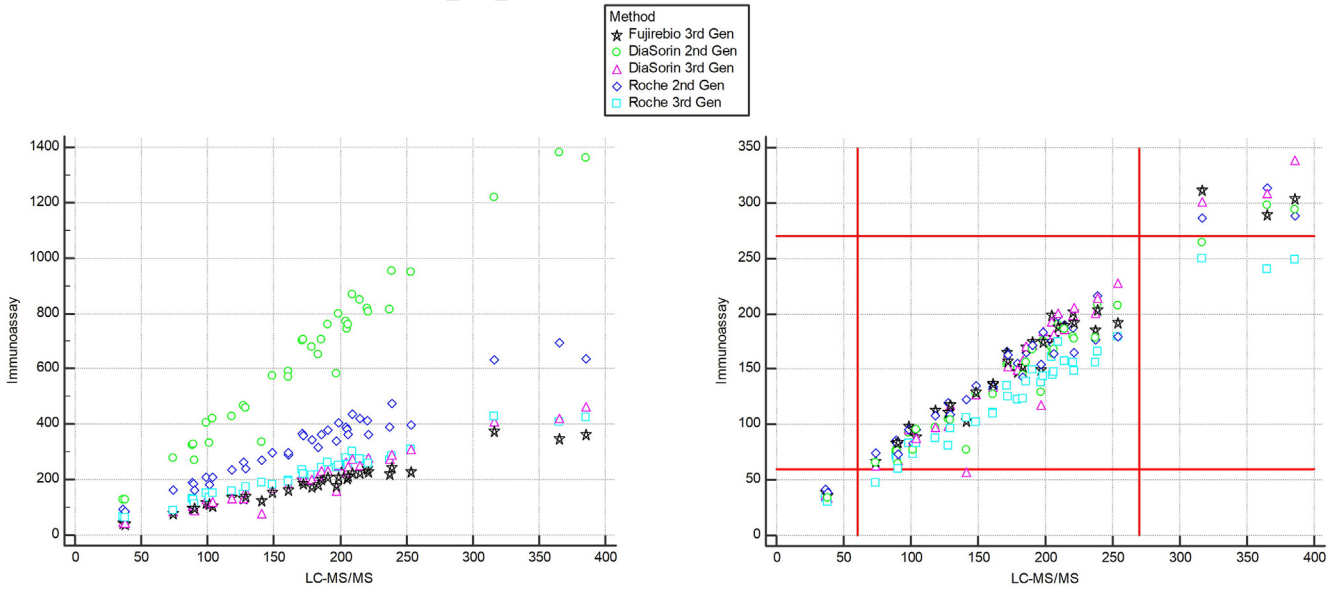


**Figure 4 | Comparison of the results obtained in hemodialyzed patients before (left) and after (right) recalibration.** The red solid bars represent 2 to 9 times the upper limit of normal (ULN) values on the liquid chromatography tandem mass spectrometry (LC-MS/MS) method, determined by the mean of the manufacturers' ULN values obtained after mathematical recalibration (measurement units are nanograms per liter). 2nd Gen, second generation; 3rd Gen, third generation.

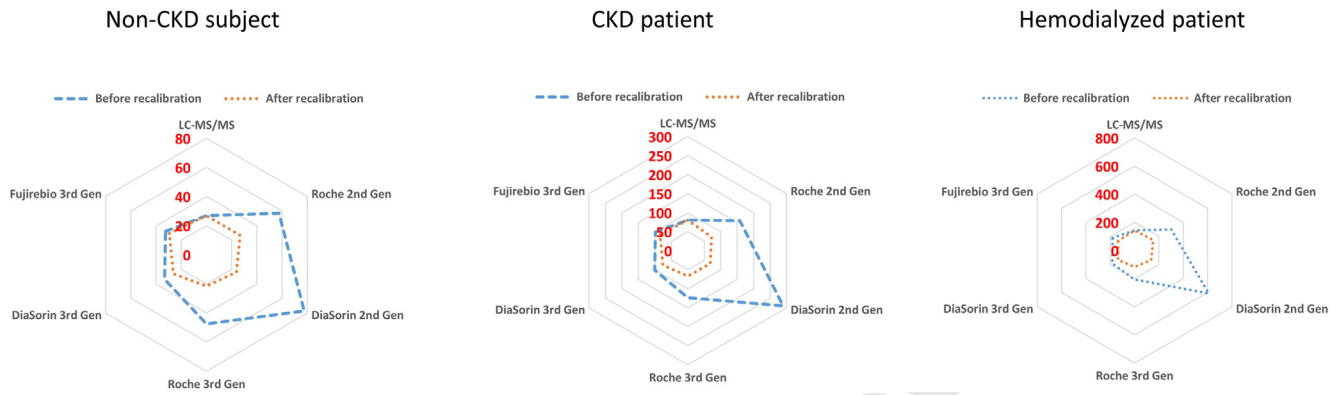


**Figure 5 | Radar charts illustrating the variations in parathyroid hormone (PTH) concentrations (measured in nanograms per liter) in 3 different subjects: a non–chronic kidney disease (CKD) subject (left), a CKD patient (middle), and a hemodialyzed patient (right).** The data presented include measurements both before (indicated by blue dashed lines) and after (indicated by orange dotted lines) the recalibration of 6 immunoassays using the reference liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The bold and red numbers along the vertical axis represent PTH concentrations (measured in nanograms per liter). 2nd Gen, second generation; 3rd Gen, third generation.

metabolism, such as the International Federation of Clinical Chemistry, Kidney Disease: Improving Global Outcomes, or the International Osteoporosis Foundation, it is noteworthy that such a study is presently lacking and has yet to be realized. As a compensatory measure, we used here a workaround—a standardized ULN value deduced from the regression equations involving PTH assays and LC-MS/MS. It is crucial to note that our intention in using this workaround was not' to preemptively institute a universal ULN value, given our awareness of potential shortcomings in manufacturer-proposed ULN values. Rather, the objective of using this method was to underscore the positive consequences of recalibrating the assays and their impact on the clinical interpretation of results, should a singular ULN value be implemented across all assays. Indeed, our results indicate that a single ULN value can be used for all recalibrated PTH assays and that recalibrated PTH assays can be used interchangeably.

One question that may arise is why this situation has persisted for more than 2 decades. As mentioned above, there are valid and invalid reasons why such differences in calibration persist despite the availability of the WHO IS. These reasons can be summarized briefly: the presence of PTH fragments that accumulate in the blood of CKD patients, exhibiting varying cross-reactivity with antibodies used in PTH assays (thus raising doubts about standardization in CKD patients); the definition of the appropriate biomarker to measure (which is nonoxidized 1–84 PTH) and the potential need of a fourth generation assay that would measure only nonoxidized PTH; the lack of higher-order reference methods, namely, an LC-MS/MS method, that offers enhanced specificity and reliability compared to immunoassays, ensuring accurate measurements; and the absence of a robust higher-order commutable reference material, capable of mimicking true human samples, that could facilitate the calibration of all assays.

In the past 2 years, remarkable advancements have been achieved in the standardization of PTH assays, particularly with the introduction of higher-order methods by Kritmetapak *et al.*[13] and Farré-Segura *et al.*[17] These groundbreaking studies have provided crucial insights, revealing that oxidized 1 to 84 PTH, previously believed to be predominantly present in the bloodstream of HD patients according to some authors,[25,26] is actually absent. Moreover, the findings from these articles have corroborated earlier research conducted using mass spectrometry, which demonstrated the absence of the 7 to 84 PTH fragment in human blood.[27,28] This fragment, known to cause cross-reactivity with second generation assays, has been confirmed to be nonexistent in the blood samples of HD patients. Kritmetapak *et al.* also demonstrated that the circulating PTH fragments they identified in the blood of CKD patients did not interfere with the Roche second generation assay, confirming the earlier intuition of Souberbielle *et al.* that a correction factor could be applied to PTH results to improve harmonization.[29] Finally, the methods developed by Kritmetapak *et al.*[13] and Farré-Segura *et al.*[17] are both calibrated against the WHO 95/646 IS and hold promise as candidate reference methods for PTH in the future against which all immunoassays could be calibrated. However, it is worth noting that the former approach, using a high resolution and not a triple quadrupole instrument, still needs some improvement in handling lower 1 to 84 PTH concentrations.

The present study provided further evidence that differences between assays are mostly due to calibration and that standardizing calibration of PTH assays is feasible. By recalibrating 5 PTH immunoassays (2 second generation and 3 third generation assays) using a set of plasma pools with established concentrations using our candidate reference measurement procedure LC-MS/MS, we achieved highly comparable PTH results in 2 different cohorts of

CKD: non-CKD and HD patients. Moreover, we demonstrated that the circulating PTH fragments did not hinder this recalibration process.

However, it is important to note that in the case of specific diseases, such as parathyroid carcinoma,[30] where post-translationally modified variants could be expressed, or when PTH monitoring is performed during parathyroid surgical resection,[31] atypical forms and variants may appear in patients' blood. These unique forms may be detectable using second generation PTH assays but not necessarily with third generation assays, or vice versa. At this point in our understanding, it is crucial to acknowledge that some of these forms might not have been identified through the HR-MS approach. With the help of previous high-performance liquid chromatography fractionation techniques, Nguyen-Yamamoto *et al.* identified 2 peaks different from 1 to 84 PTH in certain patients with parathyroid adenoma, although not in all cases.[32] This was further corroborated by Yamashita *et al.*,[9] who observed variations in PTH reactivity between the adenoma and the contralateral internal jugular vein in some patients with primary hyperparathyroidism. Exploration of such fragments or modified forms and their potential role in physiopathology will deserve further research.

In this study, we used 2 second generation PTH assays and 3 third generation assays. Yet, our findings warrant further validation through assessment using alternate second generation PTH immunoassays. Indeed, our approach here centered on optimizing sample volumes, with particular emphasis on minimizing inherent dead volume in automated immunoassay analyzers. To this end, we prioritized manufacturers who facilitated the application of second and third generation PTH assays on their platforms, specifically Roche and DiaSorin. Although the Roche second generation assay boasts global utilization, it is' worth acknowledging that the remaining assays incorporated in this study might encompass a comparatively narrower distribution volume. As such, a comprehensive evaluation across a spectrum of second generation PTH immunoassays remains a prudent next step to reinforce the robustness of our findings. Although this article represents a significant step toward improving PTH measurement, several aspects still require further work. First, we need to demonstrate that the different candidate methods yield consistent results to build a network of calibration reference laboratories. Second, the current WHO 95/646 IS is expected to be replaced with a newer IS fit-for-purpose uncertainty and IS-traceable certified values determined with a higher-order reference measurement procedure. Finally, nephrologists, laboratory specialists, and also all clinicians involved in bone and mineral metabolism must collaborate to convince manufacturers to recalibrate their assays with the higher-order reference standards. Although this endeavor would undoubtedly require substantial effort, achieving reconciliation between second and third generation assays would be a significant success. The major difference only between the 2 generations of assays would thus become the recognition or not of the amino-PTH, a post-translationally modified form of PTH overproduced in parathyroid carcinoma and in severe forms of secondary hyperparathyroidism observed in some HD patients.[30,33] Even if limited to 5 PTH assays, the successful demonstration of the recalibration results presented here serves as a proof of concept that recalibration of PTH assays is feasible. However, translating this concept into practical implementation will undoubtedly present challenges that can hopefully be overcome through close collaboration among the involved stakeholders.

In conclusion, even if further work is needed, there are good reasons to think that the standardization in PTH measurement is feasible, ultimately benefiting the care of patients.

## DISCLOSURE

EC is a consultant to DiaSorin, IDS, Fujirebio, Roche Diagnostics, Snibe, Nittobo, and bioMérieux. PD is a consultant to IDS. CLG is a consultant to Roche Diagnostics.

## DATA STATEMENT

The raw data presented in this study are available on Mendeley Data: https://data.mendeley.com/datasets/skfsbx87b7/1.

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