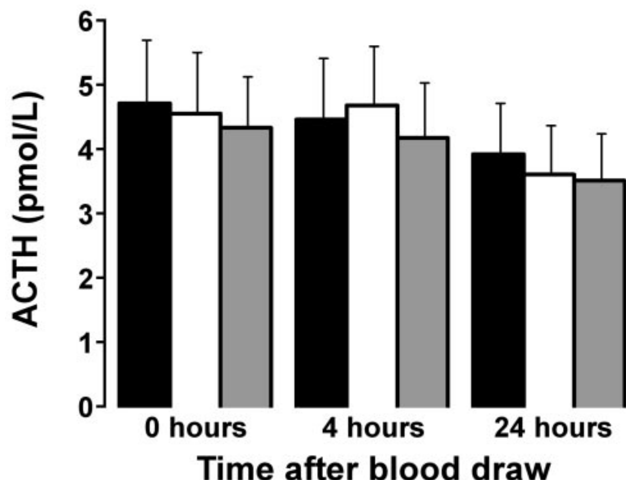

Fig. 1. Stability of ACTH in glass and plastic tubes

Mean ACTH concentrations in EDTA plasma collected in glass tubes (■), plastic tubes (□), and siliconized glass tubes (▨). Plasma was separated immediately (0 h) after the blood draw, or after 4 and 24 h storage of the blood samples at 4 °C (n = 31). *Error bars* correspond to 95% confidence intervals of the means. There were no statistically significant differences between tube types at any time point. All tube types gave significantly lower readings at 24 h than at 4 h and at baseline ($P < 0.0001$).

Monoject, and Greiner Vacuette are comparable to Becton Dickinson glass SS tubes when used to collect specimens for endocrine testing. There may be minor differences among the three brands, but they are unlikely to be clinically significant. Some analytes, such as ACTH, are inherently unstable, but this is not accentuated by plastic tubes. Conversely, there is also no evidence for the widely held belief (quoted in the specimen requirement instructions of many laboratories) that significant amounts of ACTH adsorb to nonsiliconized glass tubes. To improve ACTH stability in either tube type, it appears likely that further measures, such as addition of potent protease inhibitors, might have to be taken; however, this lies outside the scope of our study.

There are a few minor provisos, however. Storage of serum in plastic gel tubes for long periods may alter CA-125 results, but after 1 week at 4 °C, such changes are usually not clinically significant. There may be some other analytes that could be affected similarly or worse, and stability studies should probably be performed for any analyte that is considered for long-term storage. Another issue is that for three analytes (PTH, IGF-I, and OHPG) our study did not include samples that contained analyte concentrations above the upper limit of the reference interval. It is conceivable, but unlikely, that plastic tubes may behave differently at higher analyte concentrations.

Finally, plastic is the better specimen container to reduce interference from hemolysis in clinical testing, which is particularly relevant to insulin measurements but may affect other tests.

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Assessment of Parathyroid Function in Clinical Practice: Which Parathyroid Hormone Assay Is Better? *Stefano A. Santini,[1] Cinzia Carrozza,[1] Carlo Vulpio,[2] Ettore Capoluongo,[1] Giovanna Luciani,[2] Paola Lulli,[1] Bruno Giardina,[1] and Cecilia Zuppi[1*]* ([1] Institute of Biochemistry and Clinical Biochemistry, Hormone Research Unit, and [2] Institute of Surgical Clinic, Hemodialysis Unit, Catholic University School of Medicine, Rome, Italy; * address correspondence to this author at: Institute of Biochemistry and Clinical Biochemistry, Catholic University School of Medicine, Largo F. Vito 1, 00168 Rome, Italy; fax 039-6-30151918, e-mail czuppi@rm.unicatt.it)

Parathyroid hormone (PTH) is a single-chain 84-amino acid polypeptide synthesized by the parathyroid glands. In the blood it is thought to circulate as a mixture of whole molecule [PTH (1–84)] and N- and C-terminal (C-PTH) fragments produced in the parathyroid glands and liver *(1, 2)*. In patients with intact renal function, the non-(1–84) PTH, identified by HPLC, reportedly accounts for ~21% of PTH(1–84) in hypercalcemia and ~10% in hypocalcemia *(3)*. C-PTH fragments accumulate in renal failure up to 40–50% of total PTH *(4)* and may be implicated in the PTH resistance observed in these patients. It is not known whether these fragments can mimic the biological effects of PTH(1–84) or, in contrast, react with distinct receptors *(5–8)*.

The major large C-PTH fragment with partially preserved N-terminal structure is PTH(7–84), often considered to be the likely cross-reacting peptide in "intact PTH" (I-PTH) assays *(6–9)*. The biological activity of this

fragment is not definitively known *(10–13)*. The large increase of C-PTH fragments in renal failure may complicate monitoring of patients *(14, 15)*.

Determination of PTH has also been reported as predictive of different forms of renal osteodystrophy *(14)*. In most laboratories, I-PTH assays from several manufacturers are routinely performed, although the cutoff for PTH concentrations in the classification of adynamic bone in dialysis patients is still controversial *(5–7)*. These assays use antibodies against amino acids 15–34 and 50–65 of the PTH molecule and, thus, also measure C-PTH fragments with preserved N-terminal structure [such as PTH(7–84)]. A newly available (Bio-Intact) PTH assay measures only the "whole" molecule (residues 1–84) because the antibodies used recognize epitopes in the regions of amino acids 1–5 and 50–65 *(16)*. This assay thus appears similar to that proposed by Gao et al. *(17)*, which uses antibodies against the regions of amino acids 1–4 and 39–84.

We measured PTH by two immunometric assays, intact PTH (Roche), which hypothetically cross-reacts with PTH fragments, and whole PTH (Nichols Bio-Intact PTH), which does not react, in serum samples from three groups: 75 patients (40 males and 35 females) with chronic renal failure on maintenance therapy at our Hemodialysis Unit, 30 patients (18 males and 12 females) with primary hyperparathyroidism (PHPT), and 33 healthy individuals (18 males and 15 females). The mean (SD) ages of the study participants were 48 (15), 46 (13), and 44 (7) years, respectively, for the three groups. Hemodialysis was performed three times weekly for a mean (SD) of 4 (0.3) h. Patients were treated with oral calcium- and/or phosphate-chelating agents according to DOKI guidelines. PHPT patients had normal renal function. Blood samples, collected by venipuncture at 0800 in the morning, were centrifuged at 1500*g*, and sera were kept at −70 °C and thawed only once for PTH measurement by the two PTH assays on the same day. All samples had been obtained after receipt of informed consent and local ethics committee approval.

The whole-PTH chemiluminescence immunoassay [Bio-intact PTH(1–84) assay] uses an acridinium ester-labeled goat anti-PTH polyclonal antibody, which binds to the first five N-terminal amino acids of the human PTH molecule, and a biotinylated capture polyclonal antibody that binds at amino acids 57–62. The determinations were performed on a Nichols Liaison Advantage®. The intraassay CV was 3.8% and the interassay CV was 5.1% at concentrations of 25.0 and 145.0 ng/L, respectively.

The intact-PTH electrochemiluminescence immunoassay (Roche Intact PTH) uses a biotinylated monoclonal antibody, which reacts with amino acids 26–32, and a capture ruthenium-complexed monoclonal antibody, which reacts with amino acids 55–64. The determinations were performed on Roche Modular E 170®. The intraassay CV was 4.1% and the interassay CV was 5.8% at concentrations of 35.0 and 180.0 ng/L, respectively.

We measured scalar dilutions of Roche Intact PTH calibrator 2, which had a reported concentration of 3700 ng/L, and of Nichols Bio-intact PTH (1–84) calibrator B, which had a reported concentration of 1210 ng/L, on both instruments.

All statistical calculations were performed with GRAPHPAD PRISM Software (Graphpad Software Inc.), except for the Deming regression [EP-Suite 9-A for Windows™ *(18)*], which was done with EP Evaluator (D.G. Rhoads Associates, Inc.).

The main methodologic differences of the two studied methods are presented in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue7/.

The Deming regression analyses comparing intact and whole PTH from the three groups are shown in Fig. 1. In uremic patients (Fig. 1A), the slope was 0.54 (R = 0.97), indicating that values obtained by the whole-PTH assay were significantly (P <0.0001) lower (~ 46%) than those obtained by the intact-PTH assay. Thus, uremic patients may have approximately equal plasma concentrations of PTH(1–84) and PTH fragments. When we divided the uremic population into two subgroups with I-PTH values greater than or less than 200 ng/L, the regression parameters were little changed (data not shown). For the group of healthy individuals, the difference between the two assays was 36% (P <0.0001; Fig. 1B; slope = 0.64; R = 0.91), and for PHPT patients, the difference between assays was 24% (P <0.0001; Fig. 1C; slope = 0.76; R = 0.95).

ANOVA indicated mean (SD) ratios of 1.41 (0.17) for healthy individuals (P <0.001; R = 0.91), 2.0 (0.41) for uremic patients (P <0.001; R = 0.98), and 1.26 (0.28) for PHPT patients (P <0.01; R = 0.90), consistent with the differences of the respective slopes. Moreover, as evidenced by the correlation coefficients (R), the differences between the "whole-molecule" and "intact" measurements were constant, along the entire measuring range, within a group of patients.

Scalar dilutions of Roche Intact PTH calibrator 2 (stated concentration, 3700 ng/L), analyzed on the Roche Modular E-170, gave the expected values (Table 1), but gave higher values on the Nichols Liaison Advantage (mean of 28% higher; P <0.05). Scalar dilutions of Nichols calibrator B (stated concentration, 1210 ng/L) on the Liaison Nichols Advantage gave the expected values, but lower results, by as much as 50% of the expected values (P <0.05), when analyzed on the Roche Modular E-170.

Our data confirm the reports in the literature of high correlation and a linear relationship between the methods *(19, 20)*, with lower results by the whole-PTH [PTH(1–84)] assay in all three patient groups. If the differences between the two methods are to be ascribed solely to the presence of C-PTH fragments, N-terminally truncated PTH fragments should represent ~36% of PTH(1–84) in healthy individuals, 24% in patients with PHPT, and 46% in uremic patients.

The slopes of the three regression equations are rather different, as evidenced by their almost nonoverlapping confidence intervals (Fig. 1). This would suggest that the presence of fragments is rather evident, even if in different amounts, in all three groups of patients. If it is well

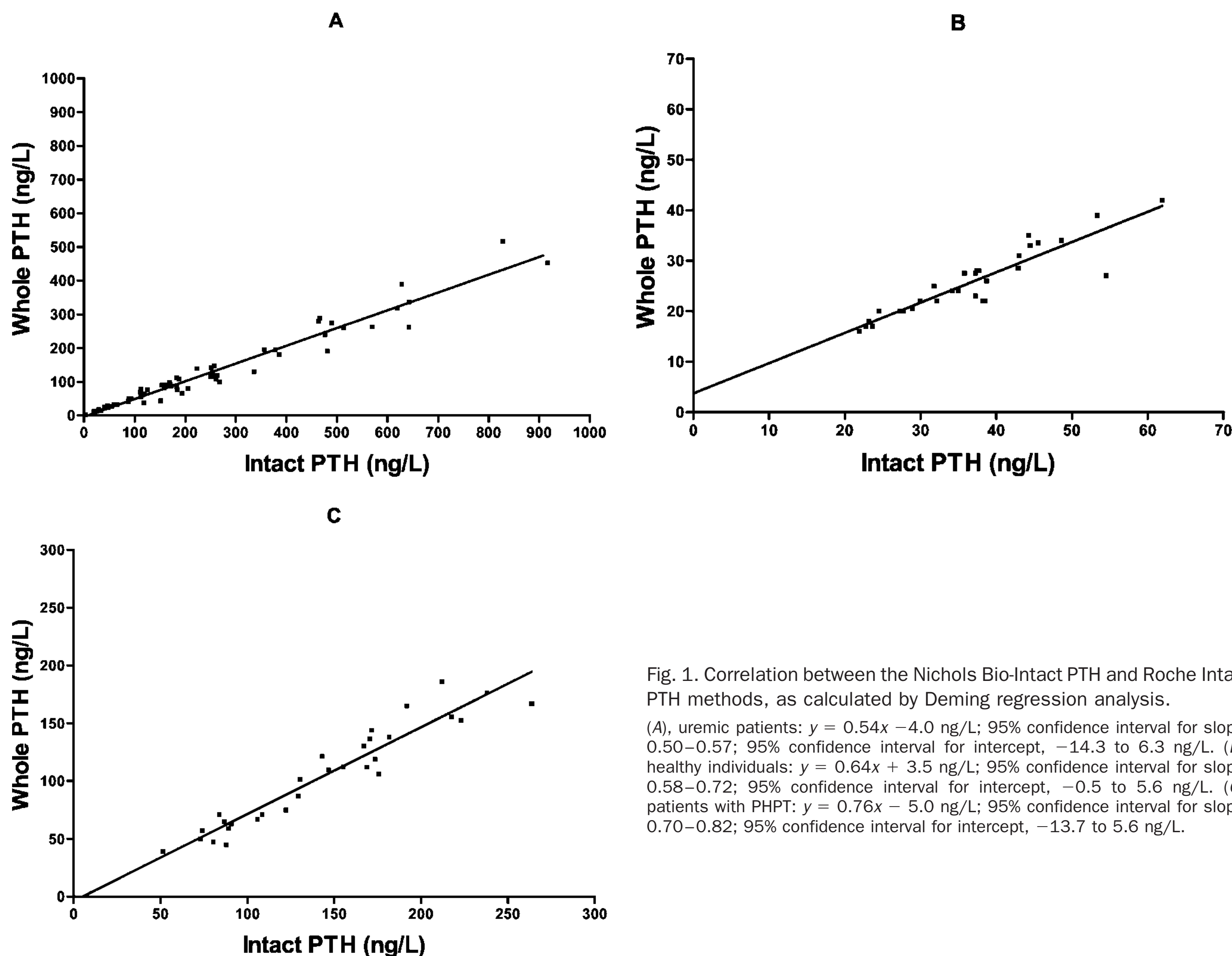

Fig. 1. Correlation between the Nichols Bio-Intact PTH and Roche Intact PTH methods, as calculated by Deming regression analysis.

(*A*), uremic patients: $y = 0.54x - 4.0$ ng/L; 95% confidence interval for slope, 0.50–0.57; 95% confidence interval for intercept, −14.3 to 6.3 ng/L. (*B*), healthy individuals: $y = 0.64x + 3.5$ ng/L; 95% confidence interval for slope, 0.58–0.72; 95% confidence interval for intercept, −0.5 to 5.6 ng/L. (*C*), patients with PHPT: $y = 0.76x - 5.0$ ng/L; 95% confidence interval for slope, 0.70–0.82; 95% confidence interval for intercept, −13.7 to 5.6 ng/L.

known that uremic patients have a relevant amount of fragments because of their lower clearance, whereas the detection of fragments in distinct amounts appears somewhat ambiguous in the two other study groups.

We hypothesized that our results cannot be attributed solely to the presence of C-terminal fragments of PTH. In fact, the calculated ratio for the Liaison and Modular data (see Table 1) varied from 1.21 to 1.42 in the dilution study with Roche calibrator 2 and from 1.48 to 1.95 in the study with Nichols calibrator B. This indicates a nonconstant ratio for the two calibrators as well as a decrease with dilution. These phenomena could be related to nonequivalent calibration curves and/or matrix differences between the calibrators for the two assays *(16)*, confirm-

Table 1. Results for scalar dilutions of Roche calibrator 2 measured on Modular E-170 and on Liaison Advantage and of Nichols calibrator B measured on Liaison Advantage and on Modular E-170.[a]

Roche Calibrator 2			Nichols Calibrator B		
Modular		Liaison	Liaison		Modular
Expected, ng/L	Found, ng/L	Found, ng/L	Expected, ng/L	Found, ng/L	Found, ng/L
115	126	153[b]	75	89	60[b]
230	234	333[b]	150	171	103[b]
462	469	655[b]	435	503	272[b]
925	928	1264[b]	907	852	493[b]
3700	3613	>1800	1210	1188	608[b]

[a] Results are for triplicate measurements.

[b] $P < 0.05$, Student *t*-test for paired data.

ing the observed differences between the two methods. Our study is even more intriguing if we consider that, very recently, a new molecular form of PTH, with structural integrity of the PTH(1–4) region and a modified PTH(15–20) region, has been identified by HPLC in primary and secondary hyperparathyroidism *(21)*. In fact, this newly discovered form of PTH appears to be immunoreactive and detectable by the whole-PTH assay but not by the intact-PTH assay.

In conclusion, the unexpected constant differences in PTH values among immunoassays, observed in both uremic and PHPT patients and in healthy individuals, could be attributable to the different calibration procedures in addition to the presence of PTH fragments. It would be useful for manufacturers to reduce the systemic variability among methods by use of a more standardized method of calibration and use of antibodies that recognize the only biologically active PTH molecule.

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Protein Bead Array for the Detection of HIV-1 Antibodies from Fresh Plasma and Dried-Blood-Spot Specimens, *Sylvie Faucher,[1*] Alexandre Martel,[1] Alice Sherring,[1] Tao Ding,[1] Laurie Malloch,[2] John E. Kim,[2] Michèle Bergeron,[1] Paul Sandstrom,[3] and Francis F. Mandy[1]* ([1] National HIV Immunology Laboratory, National HIV and Retrovirology Laboratories, Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, ON, Canada; [2] National Laboratory for HIV Reference Services, National HIV and Retrovirology Laboratories, Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, ON, Canada; [3] National HIV and Retrovirology Laboratories, Centre for Infectious Disease Prevention and Control, Health Canada, Ottawa, ON, Canada; * address correspondence to this author at: National HIV Immunology Laboratory, National HIV and Retrovirology Laboratories, Centre for Infectious Disease Prevention and Control, Bldg. 6, P.L. 0603B1, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2; fax 613-946-3237, e-mail sylvie_faucher@hc-sc.gc.ca)

Simultaneous multianalyte immunoassays offer advantages, including reduction of technical operations, execution time, and overall costs. The suspension array technology (SAT), which uses microfluorospheres with flow cytometry, is a multianalyte immunoassay that requires small specimen volumes, such as those obtained by less-invasive blood sampling approaches such as heel or finger sticks, as well as those obtained in pediatric specimens. The dried-blood-spot (DBS) technology has become an important screening tool for clinical and epidemiologic testing *(1–3)*. It is particularly convenient in rural, resource-limited settings where trained personnel and adequate facilities for blood collection, processing, transport, and storage may not be available. This approach, however, provides limited specimen volume (~5–6 μL of serum/6-mm punch) *(2)*. Fortunately, SAT is well suited to support such a format *(4, 5)*.

The range of applications for SAT includes antibody, oligonucleotide, peptide, and protein bead arrays (PBAs) *(4, 6–17)*. PBAs have been used for the simultaneous detection of serum antibodies to infectious agents for systemic candidiasis *(18)*, herpes viruses *(19)*, measles, mumps *(20)*, and HIV *(21, 22)*. The successful detection of