Supplemental Table 1. PTH peptide description, purity, and net peptide content.

Compound Name	Manufacturer	Purity (HPLC-UV)	Net Peptide Content (AAA)	Neutral m/z	Amino Acid Sequence
PTH1-84	NIBSC	93.00%	90.91%	9418.96	SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH7-84	GoldBio	98.70%	60.08%	8775.65	LMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH28-84	Innovagen	95.49%	94.13%	6175.25	LQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH34-77	Innovagen	95.66%	86.66%	4712.46	FVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNV
PTH34-84	Innovagen	95.43%	82.49%	5468.91	FVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH37-77	Innovagen	95.88%	88.61%	4395.29	LGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNV
PTH37-84	Innovagen	95.41%	85.15%	5151.74	LGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH38-77	Innovagen	95.95%	88.67%	4282.21	GAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNV
PTH38-84	Innovagen	95.98%	25.01%	5038.65	GAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
PTH45-84	Innovagen	95.12%	85.00%	4376.27	DAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
U-^{15}N-PTH1-84	GoldBio	98.56%	99.88%	9543.58	U-^{15}N-SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
U-^{15}N-PTH7-84	GoldBio	98.44%	95.49%	8775.65	U-^{15}N-LMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ
^{13}C/^{15}N-PTH28-84	Innovagen	99.46%	46.30%	6183.25	LQDVHNFVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKA(K*)SQ
^{13}C/^{15}N-PTH34-77	Innovagen	97.99%	85.83%	4718.46	FVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVN(V*)
^{13}C/^{15}N-PTH34-84	Innovagen	99.65%	59.00%	5476.91	FVALGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKA(K*)SQ

Compound Name	Manufacturer	Purity (HPLC-UV)	Net Peptide Content (AAA)	Neutral m/z	Amino Acid Sequence
$^{13}C/^{15}N$-PTH37-77	Innovagen	98.45%	52.49%	4401.29	LGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVN(V*)
$^{13}C/^{15}N$-PTH37-84	Innovagen	99.90%	53.50%	5159.73	LGAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKA(K*)SQ
$^{13}C/^{15}N$-PTH38-77	Innovagen	99.38%	85.00%	4288.20	GAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVN(V*)
$^{13}C/^{15}N$-PTH38-84	Innovagen	99.85%	57.20%	5046.65	GAPLAPRDAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKA(K*)SQ
$^{13}C/^{15}N$-PTH45-84	Innovagen	95.27%	71.60%	4384.26	DAGSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKA(K*)SQ

*PTH peptides were assessed for purity (HPLC-UV) and net peptide content (amino acid analysis/AAA). Concentrations of stock standards were adjusted after preparation based on the net peptide content for each peptide. Neutral m/z, and amino acid sequence, including the location of labeled amino acids in the stable-isotope labeled internal standards, are described above.

Supplemental Table 2. Mass spectrometry settings used for both C-terminal and N-terminal PTH methods.

Parameter	**Setting**
Scan Type	Full MS
Polarity	Positive
Scan Range	m/z 303-800
Spectrum Data Type	Centroid
Resolution	70,000
AGC Target	3E+06
Maximum IT	200 ms
Sheath Gas Flow Rate	48
Aux Gas Flow Rate	11
Sweep Gas Flow Rate	3
Spray Voltage	3.5 kV
Capillary Temperature	275 ºC
Aux Gas Heater Temperature	300 ºC
S Lens RF Level	65

Supplemental Table 3. Characteristics of PTH peptides determined by LC-HRMS method.

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
PTH1-84	15	10.9	628.9383, 629.0049, 629.0716, 629.1383, 629.2049, 629.2716, 629.3383, 629.4049, 629.4716, 629.5383
U-^{15}N-PTH1-84	15	10.9	637.1140, 637.1808, 637.2477, 637.3146 637.3815, 637.4483 637.5152, 637.5821, 637,6489, 637.7158
PTH1-84M8(O) or PTH1-84M18(O)	15	10.7	630.0046, 630.0712, 630.1379, 630.2046, 630.2712, 630.3379, 630.4046, 630.4712, 630.5379, 630.6046
PTH1-84M8(O)M18(O)	15	10.7	631.0709, 631.1376, 631.2042, 631.2709, 631.3376, 631.4042, 631.4709, 631.5376, 631.8042, 631.8709
PTH1-84	14	10.9	673.7905, 673.8621, 673.9337, 674.0054. 674.0770, 674.1486 674.2202, 674.2918 674.3634, 674.4350
U-^{15}N-PTH1-84	14	10.9	682.5501, 682.6218, 682.6935, 682.7651, 682.8368, 682.9084, 682.9800, 683.0517, 683.1233, 683.1949
PTH1-84M8(O) or PTH1-84M18(O)	14	10.7	674.9330, 675.0044, 675.0758, 675.1472, 675.2187, 675.2901, 675.3615, 675.4330, 675.5044, 675.5758
PTH1-84M8(O)M18(O)	14	10.7	676.0755, 676.1469 676.2183, 676.2900, 676.3612, 676.4326, 676.5040, 676.5755 676.6469, 676.7183
PTH1-34	6	10.8	686.8624, 687.0291, 687.1958, 687.3624,

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
			687.5291, 687.6958, 687.8624, 688.0291, 688.1958, 688.3624
C^{13}/N^{15}-PTH1-34	6	10.8	688.5286, 688.6952, 688.8619, 689.0286, 689.1952, 689.3619, 689.5286, 689.6952, 689.8619, 690.0286
PTH1-34	7	10.8	588.8831, 589.0260, 589.1688, 589.3117, 589.4545, 589.5974, 589.7403, 589.8831, 590.0260, 590.1688
C^{13}/N^{15}-PTH1-34	7	10.8	590.3112, 590.4541, 590.5970, 590.7398, 590.8827, 591.0255, 591.1684, 591.3112, 591.4541, 591.5970
PTH7-34	6	9.4	579.6428, 579.8095, 579.9761, 580.1428, 580.3095, 580.4761, 580.6428, 580.8095, 580.9761, 581.1428
C^{13}/N^{15}-PTH7-34	6	9.4	581.3090, 581.4756, 581.6423, 581.8090, 581.9756, 582.1423, 582.3090, 582.4756, 582.6423, 582.8090
PTH7-34	7	9.4	496.9806, 497.1234, 497.2663, 497.4092, 497.5520 497.6949, 497.8377. 497.9806, 498.1234, 498.2663
C^{13}/N^{15}-PTH7-34	7	9.4	498.4087, 498.5516, 498.6944, 498.8373, 498.9801, 499.1230, 499.2659, 499.4087, 499.5516, 499.6944
PTH7-84	15	10.1	586.0504, 586.1171, 586.1837, 586.2504, 586.3171, 586.3837, 586.4504, 586.5171, 586.5837, 586.6504
U-^{15}N-PTH7-84	15	10.1	593.8929, 593.9596,

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
			594.0262, 594.1596, 594.2262, 594.2929, 594.3596, 594.4262, 594.4929, 594.5595
PTH7-84	16	10.1	549.4852, 549.5477, 549.6102, 549.6727, 549.7352, 549.7977, 549.8602, 549.9227, 549.9852, 550.0477
U-^{15}N-PTH7-84	16	10.1	556.8375, 556.9000, 556.9625, 557.0250, 557.0875, 557.1500, 557.2125, 557.2750, 557.3375, 557.4000
PTH28-84	10	9.1	618.5325, 618.6325, 618.7325, 618.8325, 618.9325, 619.0325, 619.1325, 619.2325, 619.3325, 619.4325
C^{13}/N^{15}-PTH28-84	10	9.1	619.3319, 619.4319, 619.5319, 619.6319, 619.7319, 619.8319, 619.9319, 620.0319, 620.1319, 620.2319
PTH28-84	11	9.1	562.3939, 562.4848, 562.5757, 562.6666, 562.7575, 562.8484, 562.9393, 563.0302, 563.0211, 563.1211
C^{13}/N^{15}-PTH28-84	11	9.1	563.1206, 563.2115, 563.3024, 563.3933, 563.4842, 563.5751, 563.6660, 563.7569, 563.8479, 563.9388
PTH34-77	7	8.6	674.2163, 674.3592, 674.5020, 674.6449, 674.7878, 674.9306, 675.0735, 675.2163, 675.3592, 675.5020
C^{13}/N^{15}-PTH34-77	7	8.6	675.0730, 675.2159, 675.3587, 675.5016, 675.6445, 675.7873, 675.9302, 676.0730, 676.2159, 676.3587

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
PTH34-77	8	8.6	590.0652, 590.1906, 590.3159, 590.4413, 590.5666, 590.6919, 590.8173, 590.9426, 591.0679, 591.1932
C^{13}/N^{15}-PTH34-77	8	8.6	590.8148, 590.9398, 591.0648, 591.1898, 591.3148, 591.4398, 591.5648, 591.6898, 591.8148, 591.9398
PTH34-84	9	8.7	608.6642, 608.7754, 608.8865, 608.9976, 609.1087, 609.2198, 609.3309, 609.4420, 609.5531, 609.6642
C^{13}/N^{15}-PTH34-84	9	8.7	609.5525, 609.6636, 609.7747, 609.8858, 609.9969, 610.1080, 610.2191, 610.3302, 610.4413, 610.5525
PTH34-84	10	8.7	547.8985, 547.9985, 548.0985, 548.1985, 548.2985, 548.3985, 548.4985, 548.5985, 548.6985, 548.7985
C^{13}/N^{15}-PTH34-84	10	8.7	548.6979, 548.7979, 548.8979, 548.9979, 549.0979, 549.1979, 549.2979, 549.3979, 549.4979, 549.5979
PTH35-84	9	8.2	592.3233, 592.4344, 592.5455, 592.6566, 592.7677, 592.8788, 592.9899, 593.10108, 593.2122, 593.3233
C^{13}/N^{15}-PTH35-84	9	8.2	593.2115, 593.3226, 593.4337, 593.5449, 593.6560, 593.7671, 593.8782, 593.9893, 594.1004, 594.2115
PTH35-84	10	8.2	533.1917, 533.2917, 533.3917, 533.4917, 533.5917, 533.6917, 533.7917, 533.8917,

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
			533.9917, 534.0917
C^{13}/N^{15}-PTH35-84	10	8.2	533.9911, 534.0911, 534.1911, 534.2911, 534.3911, 534.4911, 534.5911, 534.6911, 534.7911, 534.8911
PTH37-77	7	7.8	628.9058, 629.0486, 629.1915, 629.3343, 629.4772, 629.6201 629.7629, 629.9058, 630.0486, 630.1915
C^{13}/N^{15}-PTH37-77	7	7.8	629.7625, 629.9053, 630.0482, 630.1910, 630.3339, 630.4768, 630.6196, 630.7625 630.9053, 631.0482
PTH37-77	8	7.8	550.4185, 550.5435, 550.6685, 550.7935, 550.9185, 551.0435, 551.1685, 551.2935, 551.4185, 551.5435
C^{13}/N^{15}-PTH37-77	8	7.8	551.1681, 551.2931, 551.4181, 551.5431, 551.6681, 551.7931 551.9181, 552.0431, 552.1681, 552.2931
PTH37-84	9	7.9	573.4227, 573.5338, 573.6449, 573.7560, 573.8671, 573.9783, 574.0894, 574.2005, 574.3116, 574.4227
C^{13}/N^{15}-PTH37-84	9	7.9	574.3109, 574.4220, 574.5331, 574.6442, 574.7553, 574.8665, 574.9776, 575.0887, 575.1998, 575.3109
PTH37-84	10	7.9	516.1812, 516.2812, 516.3812, 516.4812, 516.5812, 516.6812, 516.7812, 516.8812, 516.9812, 517.0812
C^{13}/N^{15}-PTH37-84	10	7.9	516.9805, 517.0805, 517.1805, 517.2805, 517.3805, 517.4805,

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
			517.5805, 517.6805, 517.7805, 517.8805
PTH38-77	7	7.9	612.7509, 612.8938, 613.0366, 613.1795, 613.3223, 613.4652, 613.6080, 613.7509, 613.8938, 614.0366
C^{13}/N^{15}-PTH38-77	7	7.9	613.6076, 613.7505, 613.8933, 614.0362, 614.1790, 614.3219, 614.4647, 614.6076, 614.7505, 614.8933
PTH38-77	8	7.9	536.2829, 536.4079, 536.5329, 536.6579, 536.7829, 536.9079, 537.0329, 537.1579, 537.2829, 537.4079
C^{13}/N^{15}-PTH38-77	8	7.9	537.0326, 537.1576, 537.2826, 537.4076, 537.5326, 537.6576, 537.7826, 537.9076, 538.0326, 538.1576
PTH38-84	9	7.6	560.8578, 560.9689, 561.0800, 561.1911, 561.3022, 561.4134, 561.5245, 561.6356, 561.7467, 561.8578
C^{13}/N^{15}-PTH38-84	9	7.6	561.7460, 561.8571, 561.9682, 562.0793, 562.1905, 562.3016, 562.4127, 562.5238, 562.6349, 562.7460
PTH38-84	10	7.6	504.8727, 504.9727, 505.0727, 505.1727, 505.2727, 505.3727, 505.4727, 505.5727, 505.6727, 505.7727
C^{13}/N^{15}-PTH38-84	10	7.6	505.6721, 505.7721, 505.8721, 505.9721, 506.0721, 506.1721, 506.2721, 506.3721, 506.4721, 506.5721
PTH45-84	7	8.0	626.1885, 626.3313, 626.4742, 626.6170,

PTH Species	Charge State (z)	Retention Time (Minute)	Theoretical m/z of Isotopologue Peaks
			626.7599, 626.9028, 627.0456, 627.1885, 627.3313, 627.4742
C^{13}/N^{15}-PTH45-84	7	8.0	627.3304, 627.4733, 627.6162, 627.7590, 627.9019, 628.0447, 628.1876, 628.3304, 628.4733, 628.6162
PTH45-84	8	8.0	548.0408, 548.1658, 548.2908, 548.4158, 548.5408, 548.6658, 548.7908, 548.9158, 549.0408, 549.1658
C^{13}/N^{15}-PTH45-84	8	8.0	549.0401, 549.1651, 549.2901, 549.4151, 549.5401, 549.6651, 549.7901, 549.9151, 550.0401, 550.1651
PTH48-84	8	7.7	517.6551, 517.7801, 517.9051, 518.0301, 518.1551, 518.2801, 518.4051, 518.5301, 518.6551, 518.7801
C^{13}/N^{15}-PTH48-84	8	7.7	518.5297, 518.6547, 518.7797, 518.9047, 519.0297, 519.1547, 519.2797, 519.4047, 519.5297, 519.6547

Supplemental Table 4. Imprecision and spiked recovery of human pooled serum using the C-terminal immunocapture LC-HRMS method.

PTH Species, Charge State	Lower Limit of Detection (pg/mL)	Lower Limit of Quantitation (pg/mL)	Intra-assay Imprecision Mean Range (pg/mL)	Percent Intra-assay CV Range	Inter-assay Imprecision Mean Range (pg/mL)	Inter-assay Range (%CV)	Recovery Range (%)	Recovery Mean (%)
1-84, z=+15	50	235	204-1,867	16-18	235-2,143	17-22	99-130	114
7-84 Z=+15	30	149	121-1,694	9-22	149-1,417	26-32	62-117	89
28-84, z=+11	45	186	202-1,177	12-24	186-1,146	19-24	70-101	99
34-77, z=+8	41	223	196-1,067	10-15	223-928	17-21	78-124	126
34-84, z=+9	39	168	167-1,422	14-25	168-1,574	15-21	97-124	105
37-77, z=+7	42	161	192-828	21-26	161-1,107	25-29	69-124	118
37-84, z=+10	41	158	171-875	12-22	158-807	18-29	84-129	106
38-77, z=+7	43	159	231-998	15-19	159-831	18-29	74-139	111
38-84, z=+9	24	56	38-156	14-22	56-512	28-31	92-117	105
45-84, z=+8	81	115	167-942	7-14	115-679	24-28	47-122	88

Supplemental Table 5. Estimated GFR thresholds for significant elevation of serum concentrations of phosphate, intact FGF23, and PTH peptides.

Parameter	**Estimated GFR Threshold (mL/min/1.73 m^2)**	**95% Confidence Interval**
Phosphate	22	19.25 to 25.51
Intact FGF23	33	26.40 to 40.05
Roche intact PTH	29	22.51 to 35.36
LC-HRMS PTH1-84	18	9.01 to 26.60
PTH28-84	22	12.56 to 46.56
PTH34-77	17	12.49 to 22.06
PTH34-84	19	13.19 to 24.20
PTH37-77	23	16.12 to 30.49
PTH37-84	17	13.36 to 20.64
PTH38-77	18	15.37 to 21.28
PTH38-84	17	13.10 to 21.64
PTH45-84	21	14.11 to 27.80

Supplemental Table 6. The correlation matrix of circulating PTH fragments detected in the study.

	PTH28-84	**PTH34-77**	**PTH34-84**	**PTH37-77**	**PTH37-84**	**PTH38-77**	**PTH38-84**	**PTH45-84**
PTH28-84	1							
PTH34-77	0.8229	1						
PTH34-84	0.6138	0.7341	1					
PTH37-77	0.6624	0.7112	0.7106	1				
PTH37-84	0.7122	0.8026	0.7781	0.8163	1			
PTH38-77	0.5327	0.6228	0.6247	0.6685	0.7891	1		
PTH38-84	0.6138	0.6177	0.8266	0.7801	0.7095	0.8736	1	
PTH45-84	0.6047	0.7419	0.8126	0.6261	0.8003	0.6257	0.7016	1

Supplemental Table 7. Changes in serum concentrations of PTH1-84 and PTH fragments in patients with CKD after 1 or 2 freeze-thaw cycles (*n* = 4).

PTH Peptide	Baseline Concentration (pg/mL) in Unspiked Serum	Concentration After 1 Freeze-Thaw Cycle (pg/mL)	Concentration After 2 Freeze-Thaw Cycles (pg/mL)	Mean Change in Analyte Concentration After 1 Freeze-Thaw Cycle (%)	95% Confidence Interval	*P* value	Mean Change in Analyte Concentration After 2 Freeze-Thaw Cycles (%)	95% Confidence Interval	*P* value
1-84	45	62	52	36%	–19.70% to 44.32%	0.2556	15%	–41.16% to 52.90%	0.6474
7-84	Not detected (<30 pg/mL)	Not detected (<30 pg/mL)	Not detected (<30 pg/mL)	–	–	–	–	–	–
37-84	234	190	123	–19%	–23.01% to 6.82%	0.2084	–47%	–68.93% to –8.898%	0.0191
38-77	87	67	63	–23%	–29.58% to –1.17%	0.0316	–28%	–36.02% to –2.85%	0.0370
38-84	31	37	38	20%	–8.65% to 33.42%	0.1999	23%	5.14% to 44.35%	0.0256
PTH Peptide	**Baseline Concentration (pg/mL) in Spiked Serum**	**Concentration After 1 Freeze-Thaw Cycle (pg/mL)**	**Concentration After 2 Freeze-Thaw Cycles (pg/mL)**	**Mean Change in Analyte Concentration After 1 Freeze-Thaw Cycle (%)**	**95% Confidence Interval**	***P* value**	**Mean Change in Analyte Concentration After 2 Freeze-Thaw Cycles (%)**	**95% Confidence Interval**	***P* value**
1-84	14,971	11,245	10,771	–25%	–34.91% to –1.58%	0.0366	–28%	–35.83% to –21.88%	0.010
7-84	19,788	18,719	14,915	–5%	–35% to 41.91%	0.8776	–25%	–51.46% to –13.26%	0.020
37-84	351	287	191	–18%	–27.67% to 8.92%	0.1818	–45%	–68.10% to –	0.008

PTH Peptide	Baseline Concentration (pg/mL) in Unspiked Serum	Concentration After 1 Freeze-Thaw Cycle (pg/mL)	Concentration After 2 Freeze-Thaw Cycles (pg/mL)	Mean Change in Analyte Concentration After 1 Freeze-Thaw Cycle (%)	95% Confidence Interval	*P* value	Mean Change in Analyte Concentration After 2 Freeze-Thaw Cycles (%)	95% Confidence Interval	*P* value
								24.26%	
38-77	75	67	86	–10%	–13.33% to 1.95%	0.0694	15%	–45.49% to 33.58%	0.8733
38-84	34	42	42	24%	–2.72% to 30.19%	0.0531	24%	4.56% to 33.47%	0.0372

Supplemental Table 8. Cross-reactivity of synthetic PTH fragment.

Matrix with Synthetic PTH Fragment Spike Amount	Roche Cobas iPTH Concentration (pg/mL)	Cross-Reactivity of PTH Fragment in Roche Cobas iPTH Immunoassay (%)
Universal Diluent unspiked/neat	15.1	–
Universal Diluent spiked with 30 pg/mL PTH28-84	16.0	3
Universal Diluent spiked with 30 pg/mL PTH34-84	14.7	0
Universal Diluent spiked with 30 pg/mL PTH35-84	15.5	1.33
Universal Diluent spiked with 30 pg/mL PTH37-84	15.1	0
Universal Diluent spiked with 30 pg/mL PTH38-84	14.9	0
Universal Diluent spiked with 30 pg/mL PTH45-84	14.9	0
Universal Diluent spiked with 30 pg/mL PTH48-84	14.8	0
Universal Diluent spiked with 3,000 pg/mL PTH28-84	14.9	0
Universal Diluent spiked with 3,000 pg/mL PTH34-84	16.1	0.03
Universal Diluent spiked with 3,000 pg/mL PTH35-84	15.1	0
Universal Diluent spiked with 3,000 pg/mL PTH37-84	15.4	0.01
Universal Diluent spiked with 3,000 pg/mL PTH38-84	15.5	0.01
Universal Diluent spiked with 3,000 pg/mL PTH45-84	15.3	0.01
Universal Diluent spiked with 3,000 pg/mL PTH48-84	15.4	0.01
Mass Spect Gold (MSG) serum unspiked/neat	2.2	–
MSG serum spiked with 30 pg/mL PTH7-84	30.3	93.67
MSG serum spiked with 30 pg/mL PTH28-84	2.4	0.67
MSG serum spiked with 30 pg/mL PTH34-84	2.3	0.33
MSG serum spiked with 30 pg/mL PTH35-84	2.5	1
MSG serum spiked with 30 pg/mL PTH37-84	2.6	1.33
MSG serum spiked with 30 pg/mL PTH38-84	2.7	1.67
MSG serum spiked with 30 pg/mL PTH45-84	2.8	2
MSG serum spiked with 30 pg/mL PTH48-84	2.6	1.33
MSG serum spiked with 3,000 pg/mL PTH7-84	3,221	107.29
MSG serum spiked with 3,000 pg/mL PTH28-84	3.6	0.05

Matrix with Synthetic PTH Fragment Spike Amount	Roche Cobas iPTH Concentration (pg/mL)	Cross-Reactivity of PTH Fragment in Roche Cobas iPTH Immunoassay (%)
MSG serum spiked with 3,000 pg/mL PTH34-84	2.7	0.02
MSG serum spiked with 3,000 pg/mL PTH35-84	2.6	0.01
MSG serum spiked with 3,000 pg/mL PTH37-84	2.3	<0.01
MSG serum spiked with 3,000 pg/mL PTH38-84	2.1	0
MSG serum spiked with 3,000 pg/mL PTH45-84	2.4	<0.01
MSG serum spiked with 3,000 pg/mL PTH48-84	2.3	<0.01
MSG serum spiked with 30 pg/mL of each PTH fragment in combination (PTH28-84, 34-84, 35-84, 37-84, 38-84, 45-84, 48-84)	6.0	1.81
MSG serum spiked with 3,000 pg/mL of each PTH fragment in combination (PTH28-84, 34-84, 35-84, 37-84, 38-84, 45-84, 48-84)	6.3	0.02

Supplemental Table 9. Summary of mass spectrometry-based studies for determination of circulating PTH fragments in human.

	Zhang et al. 2006	**Lopez et al. 2010**	**Current Study. 2020**
Method	MALDI-TOF MS or nanoLC-ESI-TOF-MS	Trypsin digestion followed by MALDI-TOF MS or SRM-MSIA	LC-HRMS
Immunocapture prior to MS analysis	C-terminal immunocapture (39-84)	C-terminal immunocapture (39-84)	C-terminal (44-84) and N-terminal immunocapture (1-37)
Use of stable isotope-labeled IS specific to PTH fragments	No	No	Yes
Number of patients	10 (6 healthy individuals receiving PTH 1-84 injection, 4 CKD patients)	24 (12 healthy individuals, 12 CKD5 patients)	221 patients (Varying eGFR)
PTH fragments detected	4 fragments (PTH34-84, 37-84, 38-84, 45-84)	PTH1-84 and 9 fragments (PTH28-84, 34-77, 34-84, 37-77, 37-84, 38-77, 38-84, 45-84, 48-84)	PTH1-84 and 8 fragments (PTH28-84, 34-77, 34-84, 37-77, 37-84, 38-77, 38-84, 45-84)
PTH7-84	Not reported	Not detected (By monitoring tryptic peptide 7-13)	Not detected (<30 pg/mL)
Serum concentration of PTH fragments in relation to eGFR	Not reported	Not reported	Reported

Abbreviations eGFR, estimated glomerular filtration rate; IS, internal standard; LC-ESI-TOF, liquid chromatography-electrospray ionization-time of flight; LC-HRMS, liquid chromatography-high resolution mass spectrometry; MALDI-TOF; matrix-assisted laser desorption/ionization-time of flight; MS, mass spectrometry; SRM-MSIA, selected reaction monitoring-mass spectrometric immunoassay.