Measuring testosterone: the power of a method on steroids

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accuracy of first and second generation testosterone assays and improvement through sample extraction

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The monitoring of antiandrogen treatment in patients with prostate cancer, investigation of hyperandrogenism in women, and evaluation of infants with ambiguous genitalia require accurate measurement of low testosterone concentrations. However, testosterone immunoassays have been shown to be inaccurate and often to overestimate testosterone concentrations in the low range [1]. A working group of the Endocrine Society recently reviewed this concern and presented several recommendations to ensure the accuracy of future testosterone testing for improvement of diagnosis and treatment of disease [2].

In the present study we evaluated the current situation with regard to the accuracy of seven testosterone immunoassays, including two second generation assays, by comparison with isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS). In addition, we investigated the possible improvement of these immunoassays by diethyl ether sample extraction.

Serum from 50 men, 50 women and 16 children (age 4-16 years) was collected, divided into aliquots, and stored (-20 °C) until analysis. All investigations conformed to the ethics standards of the Helsinki Declaration.

Total serum testosterone was measured singly (except in duplicate by ID-LC-MS/MS), within the same lot, and before and after extraction by ID-LC-MS/MS and seven immunoassays. We used a slightly adjusted version of a previously published ID-LC-MS/MS method (Quattro Premier XE, Waters) [3]. Validation showed that testosterone results were not affected by the modifications and were concordant with a reference method, GC-MS. All immunoassays are currently available, except for the second generation Architect® (Abbott) assay which will be available soon. None of the immunoassays investigated (Table 1) used an extraction step, as a prescribed procedure, prior to analysis. We extracted testosterone using diethyl ether as described previously [4]. The dried extracts were reconstituted in a matrix recommended by the manufacturer.

The results of the extracted samples were corrected for baseline testosterone concentration of the reconstitution matrix and mean extraction recovery. By ID-LC-MS/MS, the 116 individual serum samples were categorized and subsequently analyzed in two groups, < 4.0 nmol/L (n = 68) and > 4.0 nmol/L (n = 48), because the immunoassays show a nonlinear relationship over the entire measuring range. Samples with concentrations below the detection limit of the tested immunoassay or insufficient serum volume were excluded from data analysis (Table 1).

For untreated and extracted samples in the higher testosterone range, all immunoassays showed a good correlation with ID-LC-MS/MS (r > 0.92; Table 1). In the lower range, the correlation coefficients of the immunoassays ranged from 0.59 to 0.92. The best correlation and SD mean percentage bias in this range was observed in a nonautomated RIA. The performance of the Architect immunoassay shows a clear improvement compared to its predecessor (Supplemental Data Figure 1).

To investigate whether the accuracy of the different testosterone immunoassays could be improved by diethyl ether extraction, we first analyzed the recovery of the extraction method using ID-LC-MS/MS. Therefore, we measured all samples with ID-LC-MS/MS before and after sample extraction. The extraction method proved to be robust, because we found correlation coefficients of 0.97 and 0.99 for low and high concentrations, respectively. The effect of extraction on the correlation coefficient and mean percentage bias was most pronounced in the Architect I assay (Table 1). Its performance after sample extraction is comparable to the performance of its successor without extraction. Furthermore, sample extraction further improved the accuracy of the two second generation assays in the low range (Table 1, Supplemental Data Figure 1), which indicates that they still suffer from interfering compounds and their specificity can be further improved. For several immunoassays an increased bias was observed (Table 1); this could be explained by the design of the assay which is not calibrated for a purified reconstitution matrix.

The phenomenon that direct automated testosterone immunoassays give inaccurate high results in samples from women and children because of possible calibration and specificity problems has been addressed extensively in the literature. The precise nature of the interfering compounds has yet to be elucidated, although a significant cross-reactivity with DHEA sulfate has been reported for the Cobas® I and II assays [5].

In the present study we evaluated the accuracy before and after sample extraction of seven commercially available direct testosterone assays. In conclusion, in the > 4.0 nmol/L testosterone range, all immunoassays show a good correlation with ID-LC-MS/MS. The correlation coefficients for automated immunoassays were relatively poor for measurement of low testosterone concentrations (< 4.0 nmol/L) in untreated samples. Following diethyl ether sample extraction, the correlation coefficient of 6 of 7 immunoassays including the second generation assays increased. Although the accuracy of testosterone immunoassays has been improved since 2007 by the introduction of second generation assays, there is still need for further improvement.

REFERENCES

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[2] Rosner W, Vesper H. Toward excellence in testosterone testing: a consensus statement. J Clin Endocrinol Metab 2010;95:4542-48.

[3] Bui HN, Struys EA, Martens F, de Ronde W, Thienpont LM, Kenemans P, et al. Serum testosterone levels measured by isotope dilution-liquid chromatography-tandem mass spectrometry in postmenopausal women versus those in women who underwent bilateral oophorectomy. Ann Clin Biochem 2010; 47:248-52.

[4] Kane J, Middle J, Cawood M. Measurement of serum testosterone in women; what should we do? Ann Clin Biochem, 2007;44:5-15.

[5] Benton SC, Nuttall M, Nardo L, Laing I. Measured dehydroepiandrosterone sulfate positively influences testosterone measurement in unextracted female serum: comparison of 2 immunoassays with testosterone measured by LC-MS. Clin Chem 2011;57:1074-5.

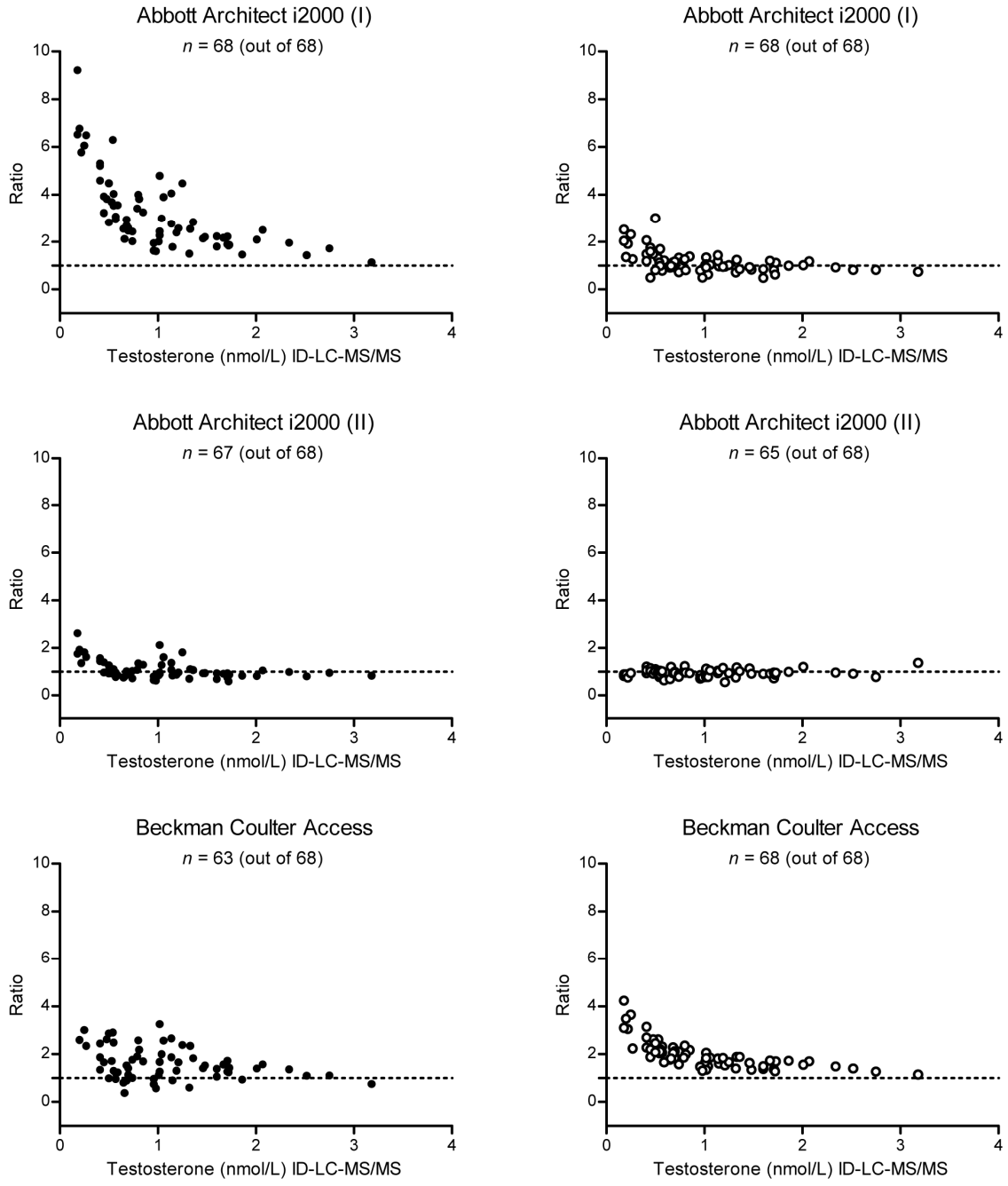
TABLE 1 (SEE NEXT PAGES): Passing-Bablok correlation analysis of 7 testosterone immunoassays against ID-LC-MS/MS for both untreated and extracted serum samples. Asterisks indicate a statistically significant difference compared with ID-LC-MS/MS. Samples with concentrations below the detection limit of the tested immunoassay or with insufficient serum volume were excluded from data analysis.

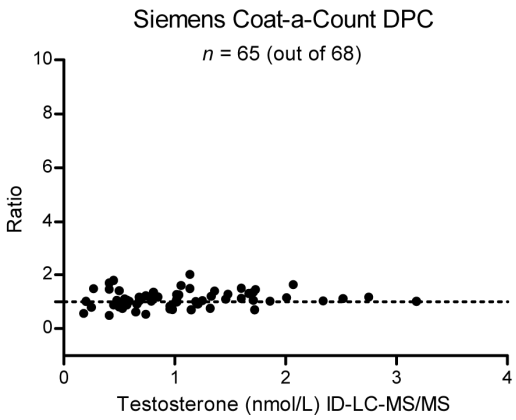
Samples < 4.0 nmol/L	*n*	intercept (nmol/L)	95% CI (nmol/L)	slope	95% CI	R	mean bias (%)	SD mean bias (%)
ID-LC-MS/MS extr.	68	0.00	-0.08 to 0.00	1.0	1.00 to 1.10	0.97	-5	14
Abbott Architect® i2000 I	68	0.75 *	0.43 to 1.05	1.79 *	1.50 to 2.20	0.71	220	157
Abbott Architect® i2000 I extr.	68	0.15	0.00 to 0.25	0.88	0.76 to 1.00	0.88	15	47
Abbott Architect® i2000 II	67	0.09	0.00 to 0.17	0.87	0.77 to 1.00	0.87	9	38
Abbott Architect® i2000 II extr.	65	0.00	0.00 to 0.03	1.00	0.93 to 1.00	0.95	-5	16
Beckman Coulter Access®	63	-0.12	-0.49 to 0.22	1.60 *	1.30 to 2.01	0.73	61	69
Beckman Coulter Access® extr.	68	0.43 *	0.36 to 0.53	1.33 *	1.21 to 1.46	0.95	97	59
Siemens Coat-a-Count®	65	-0.19 *	-0.35 to -0.08	1.29 *	1.15 to 1.50	0.92	10	31
Siemens Coat-aCount® extr.	65	-0.13	-0.27 to 0.00	1.12	1.00 to 1.29	0.93	-2	27
Siemens ADVIA Centaur®	64	0.22 *	0.01 to 0.32	1.39 *	1.17 to 1.60	0.86	59	53
Siemens ADVIA Centaur® extr.	67	-0.20	-0.55 to 0.17	2.00 *	1.67 to 2.50	0.74	107	107
Siemens Immulite® 2000	30	0.21	-0.40 to 0.60	0.79	0.50 to 1.30	0.59	18	111
Siemens Immulite® 2000 extr.	21	0.37	-0.28 to 0.74	0.77	0.56 to 1.13	0.75	0	26
Roche Cobas® II	65	-0.14	-0.40 to 0.00	1.20	1.00 to 1.46	0.79	8	45
Roche Cobas® II extr.	66	0.08	-0.07 to 0.16	1.43*	1.27 to 1.57	0.94	52	41

Samples > 4.0 nmol/L	*n*	intercept (nmol/L)	95% CI (nmol/L)	slope	95% CI	R	mean bias (%)	SD mean bias (%)
ID-LC-MS/MS extr.	48	0.05	-0.59 to 0.51	0.99	0.95 to 1.02	0.99	-2	6
Abbott Architect® i2000 I	48	0.93 *	0.27 to 1.61	0.93 *	0.88 to 0.97	0.99	0	9
Abbott Architect® i2000 I extr.	48	-1.21 *	-2.19 to -0.34	0.97	0.91 to 1.05	0.98	-12	9
Abbott Architect® i2000 II	46	-0.05	-1.44 to 1.11	1.05	0.95 to 1.14	0.97	3	12
Abbott Architect® i2000 II extr.	44	1.31 *	0.47 to 2.47	0.83 *	0.76 to 0.90	0.97	-5	11
Beckman Coulter Access®	47	1.22 *	0.61 to 1.95	0.75 *	0.69 to 0.80	0.97	-14	11
Beckman Coulter Access® extr.	46	-0.67	-1.27 to 0.10	1.25 *	1.19 to 1.30	0.99	19	12
Siemens Coat-a-Count®	47	1.20 *	0.21 to 2.05	0.86 *	0.78 to 0.94	0.97	-4	10
Siemens Coat-aCount® extr.	47	-0.22	-1.97 to 1.40	1.00	0.86 to 1.12	0.95	-2	15
Siemens ADVIA Centaur®	48	0.03	-0.72 to 0.69	0.82 *	0.78 to 0.89	0.98	-17	8
Siemens ADVIA Centaur® extr.	48	-1.72	-4.54 to 0.04	1.29 *	1.15 to 1.48	0.95	19	21
Siemens Immulite® 2000	44	0.28	-0.95 to 1.13	0.73 *	0.65 to 0.82	0.92	-27	19
Siemens Immulite® 2000 extr.	42	1.04 *	0.36 to 1.73	0.64 *	0.58 to 0.68	0.94	-35	20
Roche Cobas® II	47	0.06	-0.52 to 0.58	0.94 *	0.91 to 0.99	0.99	-4	7
Roche Cobas® II extr.	47	2.00 *	1.26 to 3.08	1.34 *	1.28 to 1.41	0.98	50	13

SUPPLEMENTAL FIGURE 1: Ratios of serum testosterone A: < 4.0 nmol/L and B: > 4.0 nmol/L by seven immunoassays compared to ID-LC-MS/MS of untreated (●) and extracted (○) samples. The x-axis represents the testosterone concentration measured by ID-LC-MS/MS (nmol/L); the y-axis represents the ratio of testosterone concentration by immunoassay divided by testosterone concentration by ID-LC-MS/MS. Samples with concentrations below the detection limit of the tested immunoassay or with insufficient serum volume were excluded from data analysis.

FIGURE 1A

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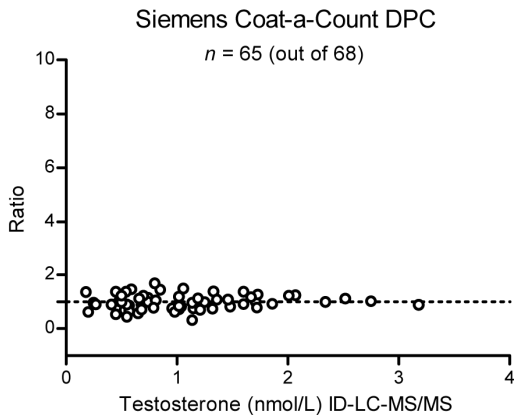
n = 65 (out of 68)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS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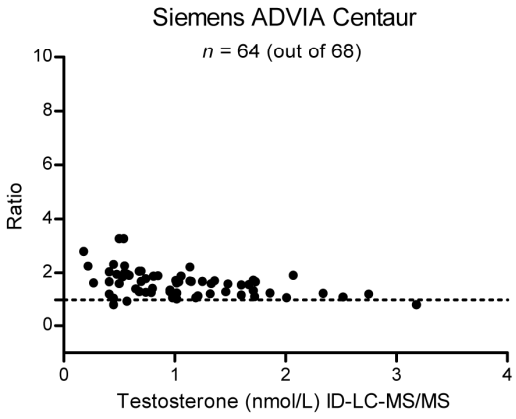
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens ADVIA Centaur
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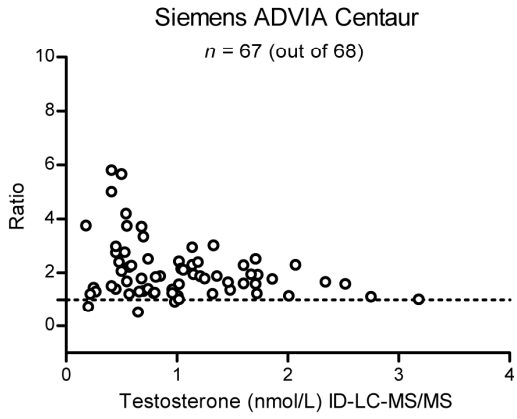
Ratio
Testosterone (nmol/L) ID-LC-MS/MS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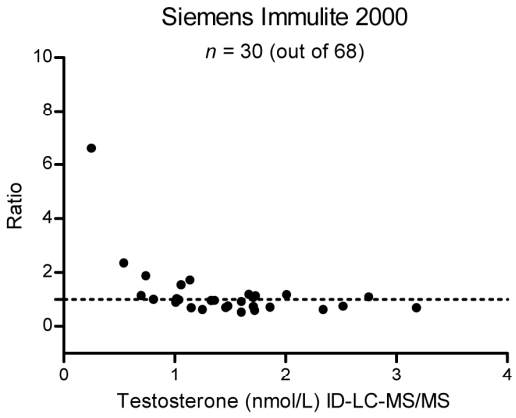
Ratio
Testosterone (nmol/L) ID-LC-MS/MS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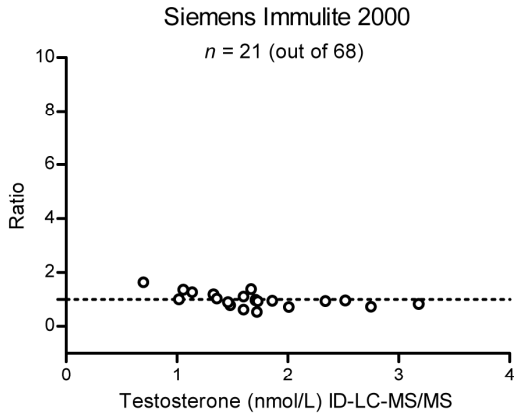
n = 30 (out of 68)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens Immulite 2000
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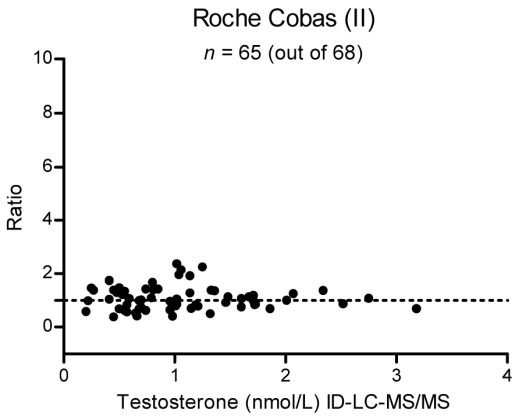
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Roche Cobas (II)
n = 65 (out of 68)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS

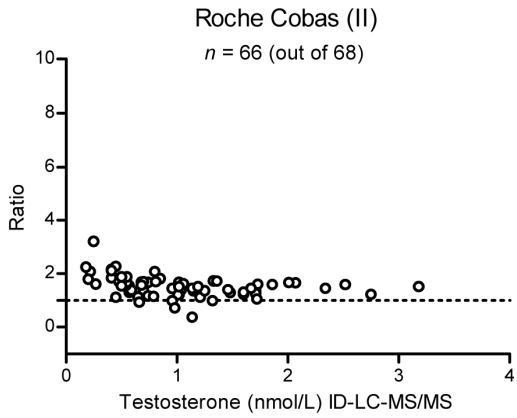
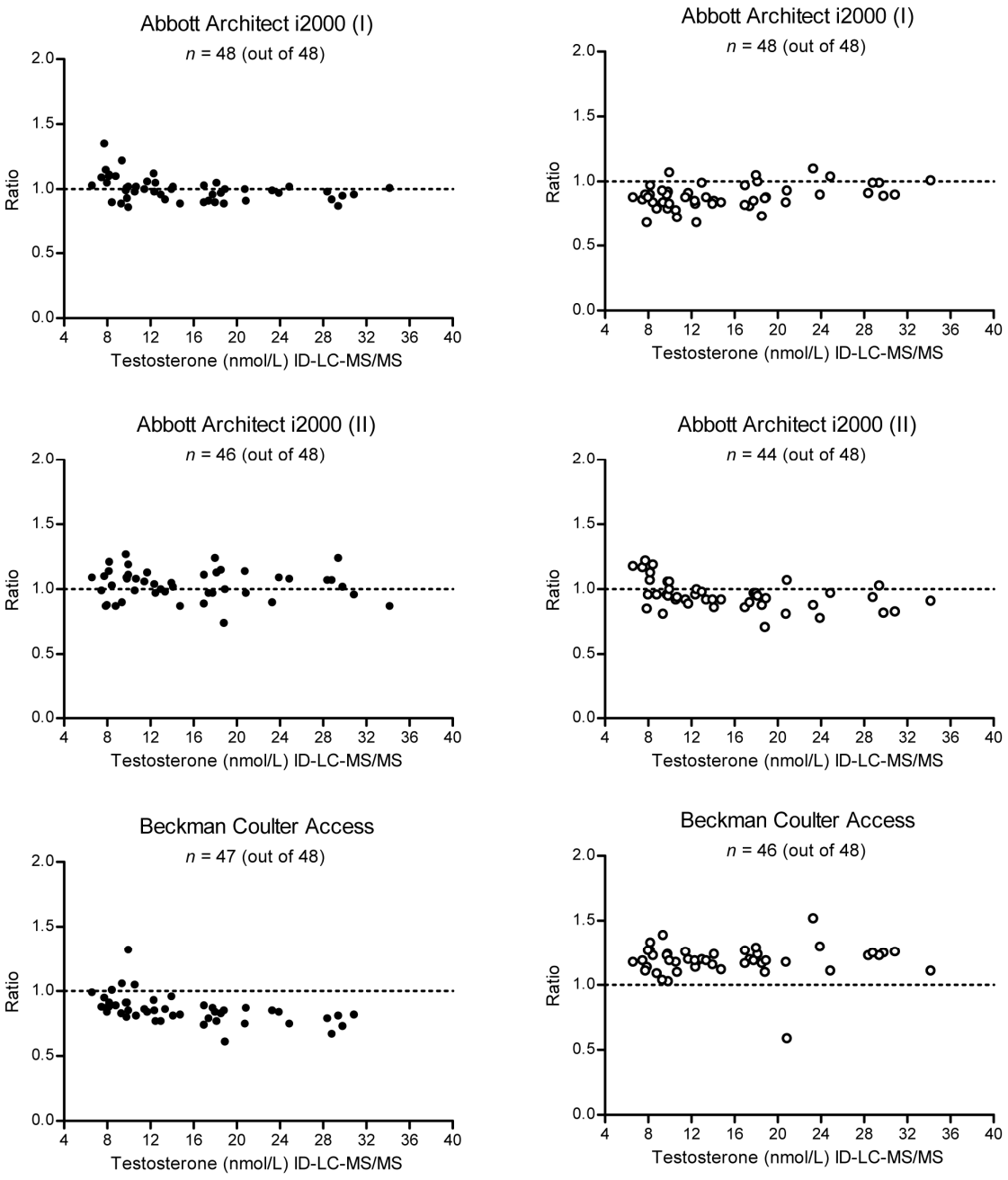
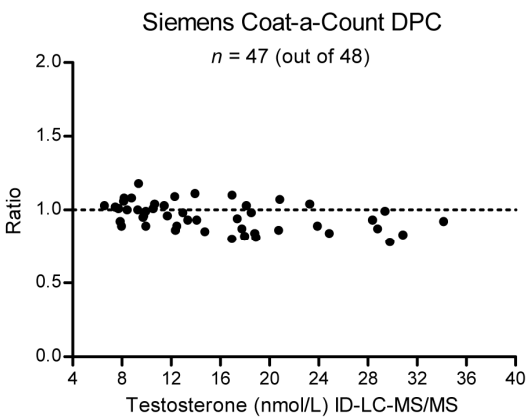
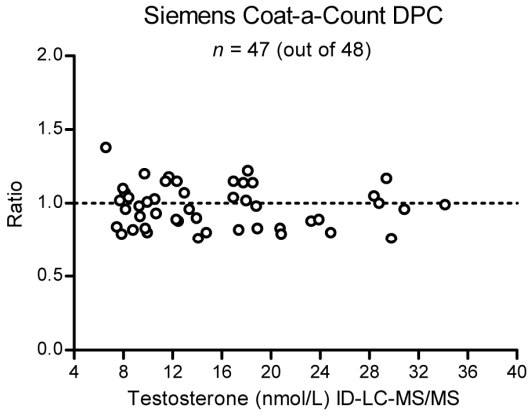
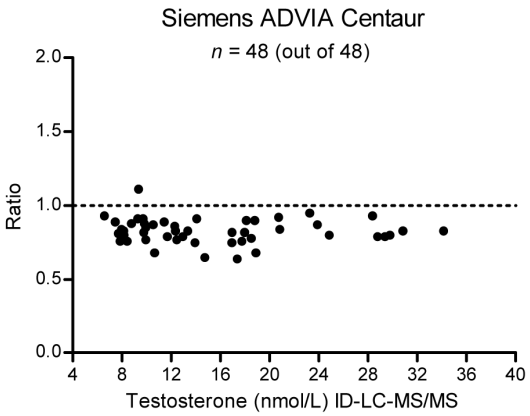

Roche Cobas (II)
n = 66 (out of 68)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS

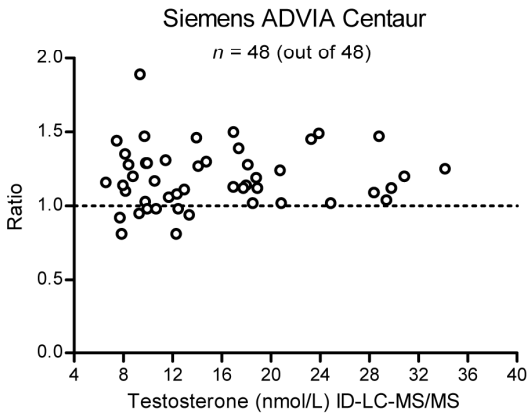
FIGURE 1B


Abbott Architect i2000 (I)
n = 48 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS
Abbott Architect i2000 (I)
n = 48 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS
Abbott Architect i2000 (II)
n = 46 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS
Abbott Architect i2000 (II)
n = 44 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS
Beckman Coulter Access
n = 47 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS
Beckman Coulter Access
n = 46 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens Coat-a-Count DPC
n = 47 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens Coat-a-Count DPC
n = 47 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens ADVIA Centaur
n = 48 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS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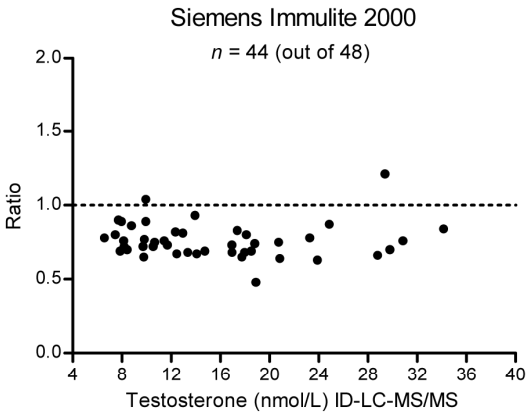
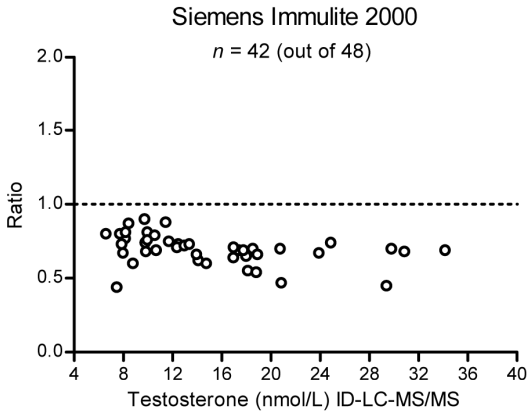
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens Immulite 2000
n = 44 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Siemens Immulite 2000
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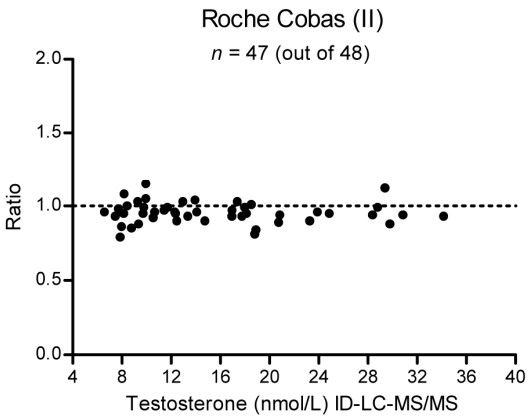
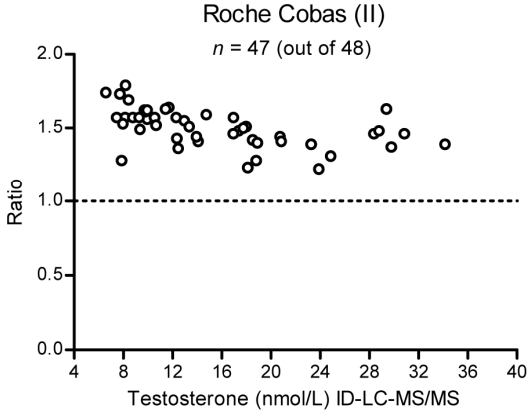
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Roche Cobas (II)
n = 47 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS


Roche Cobas (II)
n = 47 (out of 48)
Ratio
Testosterone (nmol/L) ID-LC-MS/MS