

Supplementary Data

**Sensitive LC-MS/MS Assay for Total Testosterone Quantification on Unit Resolution and High-Resolution Instruments**

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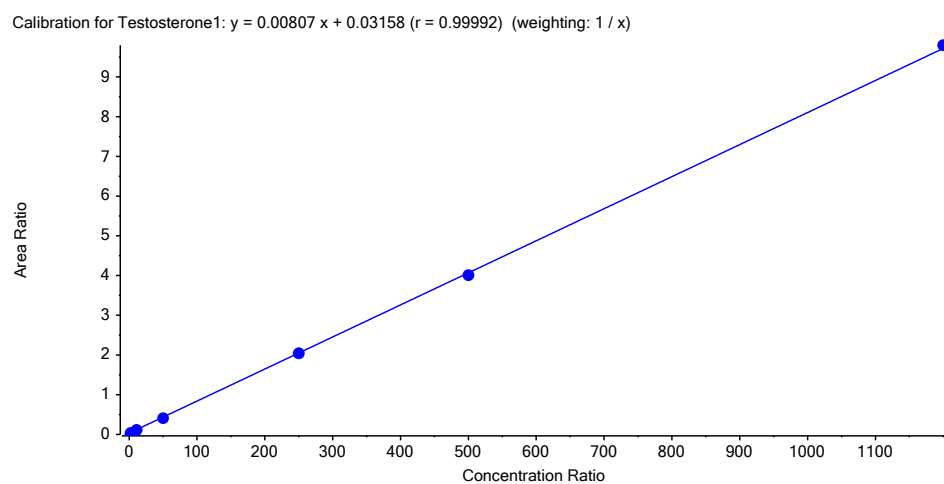
Department of Pathology and Laboratory Medicine

University of Wisconsin – Madison

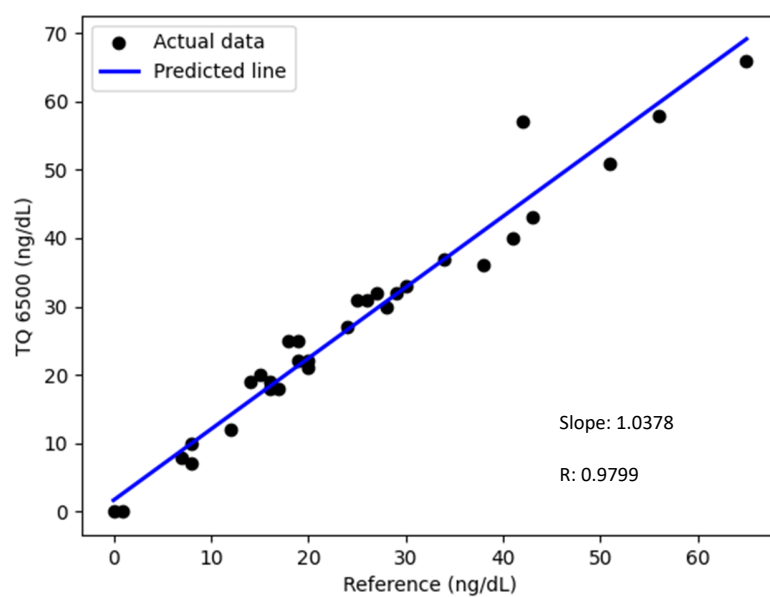
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**Supplementary Figure S1.** A six-point calibration curve with concentrations of 2, 10, 50, 250, 500, and 1200 ng/dL.



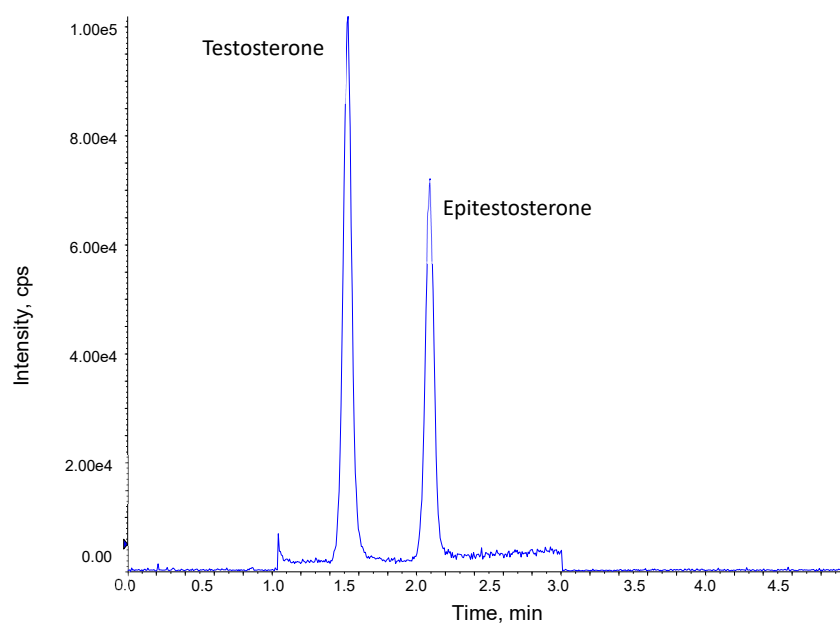
**Supplementary Figure S2.** Method comparison at a lower concentration range (< 100 ng/dL) using Deming Regression.

**Supplementary Table S1.** Hemolysis, icterus, and lipemia interference studies

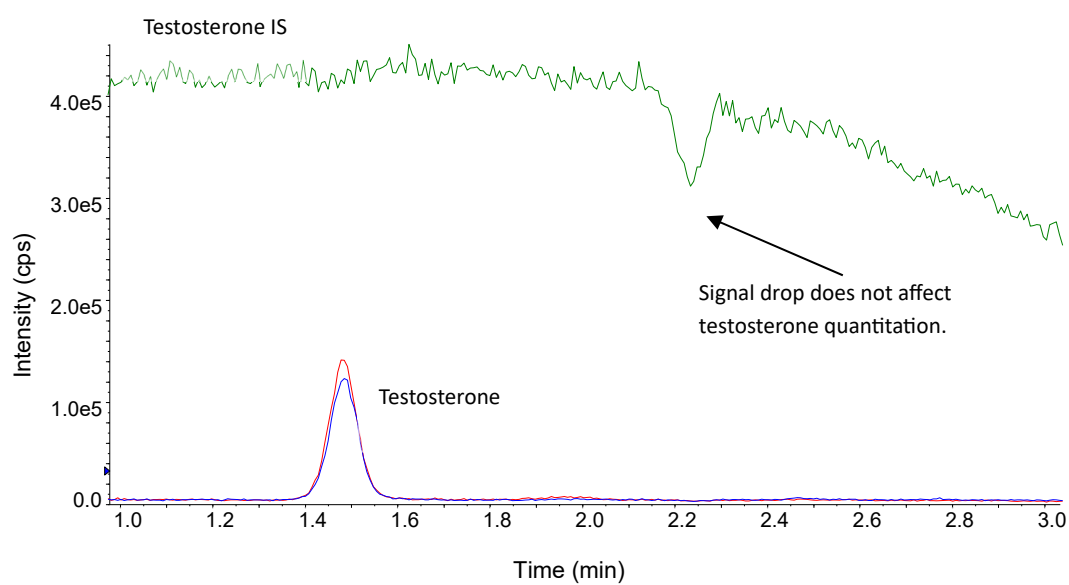
Category	Sample ID	Control (ng/dL)	Spiked (ng/dL)	Absolute Difference (ng/dL)	Relative Difference (%)
Hemolysis	S1	76	78	2	2.6
	S2	658	641	-17	-2.6
	S3	54	56	2	3.7
	S4	56	57	1	1.8
	S5	2	3	1	50
Icterus	S6	24	23	-1	-4.2
	S7	9	9	0	0
	S8	27	26	-1	-3.7
	S9	233	234	1	0.4
	S10	99	99	0	0
Lipemia	S11	52	45	7	-13.5
	S12	458	374	83	-18.1
	S13	16	15	1	-6.3
	S14	117	91	26	-22.2
	S15	82	77	5	-6.1

**Supplementary Table S2.** Accurate mass of precursor and fragment ions on OE120

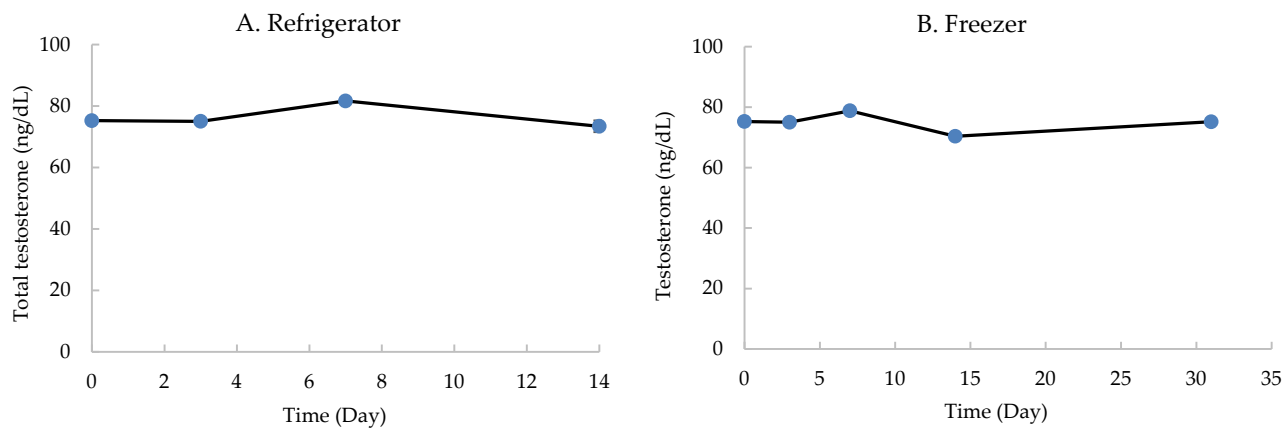
Name	Precursor	Fragment	CE
Quantifier ion	289.2162	97.0648	40
Qualifier ion	289.2162	109.065	40
IS	292.2263	100.0754	40



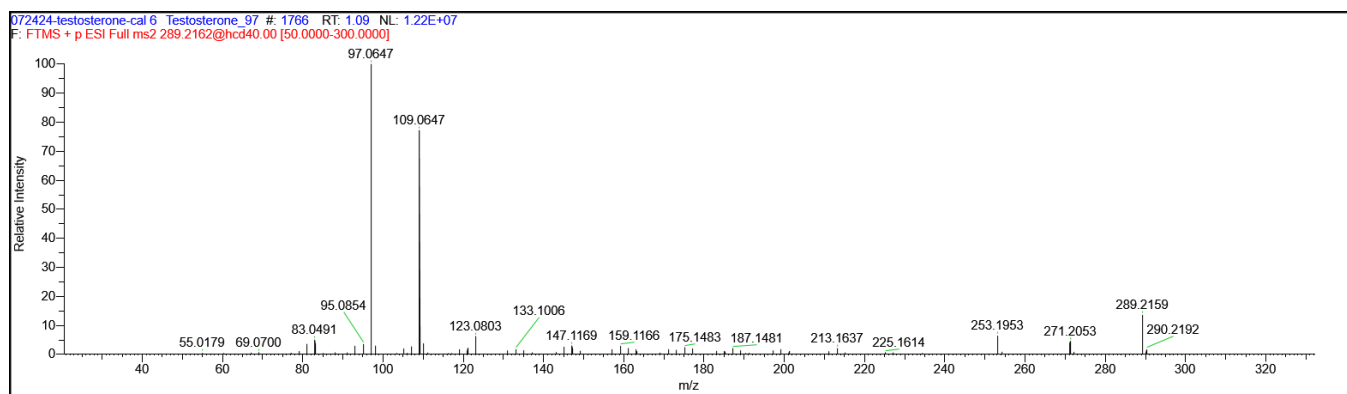
**Supplementary Figure S3.** Extracted ion chromatogram demonstrating the baseline separation of testosterone and epitestosterone.



**Supplementary Figure S4.** Matrix effect study by post-column infusion on TQ 6500



**Supplementary Figure S5.** Sample stability study after storage in refrigerator for 14 days (A) or in freezer for 31 days (B)



**Supplementary Figure S6.** High-resolution testosterone MS<sup>2</sup> spectrum collected on OE120.