Accuracy and variability of serum bioavailable testosterone testing methods

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INTRODUCTION

- **Bioavailable testosterone (BioT) includes free and albumin-bound testosterone.**
- **Total testosterone (TT) can be measured by immunoassays and LC-MS/MS, a preferred method for women and children.**
- **BioT can be calculated using various algorithms based on TT, SHBG, and albumin concentrations.**
- **It can also be measured after removal of SHBG-bound testosterone by NH_4SO_4 precipitation.**
- **The objective of the study was to compare BioT results generated by different in-house approaches with a reference lab method.**

METHODS

- **Forty nine serum samples with known BioT values (1.2 – 441.7 ng/dL) obtained from national reference laboratory.**
- **Total testosterone was measured by:**
  - **LC-MS/MS: LLE Sample Prep., Agilent 1260 HPLC, Agilent 6460 QQQ;**
  - **Electrochemiluminescence immunoassay (ECLIA): Roche cobas e 602;**
  - **Radioimmunoassay (RIA): MP Biomedicals.**
- **SHBG: ECLIA (Roche cobas e 602).**
- **BioT (NH4SO4 PPT): 50% Ammonium Sulfate Precipitation.**
- **Calculated BioT based on the following equations using fixed concentration of albumin (43 g/L).**
- **The allowable total error was ±25%.**

Table 1. Equations used for BioT calculations

Reference Lab	In House #1	In House #2	In House #3	In House #4
Modified Vermeulen	Vermeulen	Sodergard	Emadi-Konjin	Morris
Proprietary	**BioT** (mol/L) = $\{(k_{at} \times [albumin] \times [FT]/(1 + k_{at} \times [FT])\} + [FT]$ **FT** (mol/L) = $\{-b + \sqrt{(b^2 + 4a[TT]}\}/2a$ $a = k_{at} + k_t + (k_{at} \times k_t)([SHBG] + [albumin] - [TT])$ $b = 1 + k_t[SHBG] + k_{at}[albumin] - (k_{at} + k_t)[TT]$ k_t (SHBG) / k_{at} (Albumin) #1: 1×10^9 L/mol / 3.6×10^4 L/mol #2: 5.97×10^8 L/mol / 4.06×10^4 L/mol #3: 1.4×10^9 L/mol / 1.3×10^4 L/mol			**BioT** (nmol/L) = $e^{(-0.266 + 0.955 \times \ln[TT] - 0.228 \times \ln[SHBG])}$

RESULTS

Table 2. Comparison of BioT (Y_L) Calculated Using 4 Equations Based on TT (LC-MS/MS) and SHBG With Reference Lab Method (X)

Method	Correlation Equation*	Correlation Coefficient	Mean % Bias	% Bias Std Dev.
Vermeulen (Y_{L1})	Y_{L1}=0.835 X-3.46	0.997	-21.1	9.1
Sodergard (Y_{L2})	Y_{L2}=1.078 X-6.0	0.996	13.0	14.4
Emadi-Konjin (Y_{L3})	Y_{L3}=0.430 X-6.29	0.973	-68.5	8.0
Morris (Y_{L4})	Y_{L4}=0.540 X-4.02	0.969	-23.3	28.8
*Deming Regression Analysis				

Table 3. Comparison of BioT (Y_E) Calculated Using 4 Equations Based on TT (ECLIA) and SHBG With Reference Lab Method (X)

Method	Correlation Equation*	Correlation Coefficient	Mean % Bias	% Bias Std Dev.
Vermeulen (Y_{E1})	Y_{E1}=0.798 X-1.58	0.994	-15.8	31.5
Sodergard (Y_{E2})	Y_{E2}=1.034 X-5.87	0.992	19.4	36.4
Emadi-Konjin (Y_{E3})	Y_{E3}=0.405 X-3.65	0.972	-66.2	13.0
Morris (Y_{E4})	Y_{E4}=0.518 X-7.59	0.966	-23.6	34.6
*Deming Regression Analysis				

NH_4SO_4 precipitation (Y_P): Y_P=0.883X-10.93 (R=0.988), mean bias=-28.9% ±12.6%.

CONCLUSION

- **Compared to the reference lab BioT and based on the in-house LC-MS/MS TT:**
  - **The Vermeulen, Sodergard, and Morris algorithms generate values with acceptable average biases.**
  - **Both the Vermeulen and Sodergard algorithms have correlation coefficients >0.99.**
  - **The least variable methods are Vermeulen and Emadi-Konjin algorithms with a percent bias SD of 9.1% and 8.0%, respectively.**
- **Based on the in-house ECLIA:**
  - **The Vermeulen, Sodergard, and Morris algorithms also produce values with acceptable average biases.**
  - **The Vermeulen and Sodergard algorithms show correlation coefficients >0.99.**
- **In comparison with LC-MS/MS, BioT results calculated using ECLIA TT values demonstrate larger variability in biases compared to the reference lab BioT for all 4 algorithms.**
- **We select the Vermeulen algorithm and LC-MS/MS TT method for calculating BioT.**