TWO METHODS FOR SERUM FREE TESTOSTERONE MEASUREMENT: UTILITY AND LIMITATIONS

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1 Background

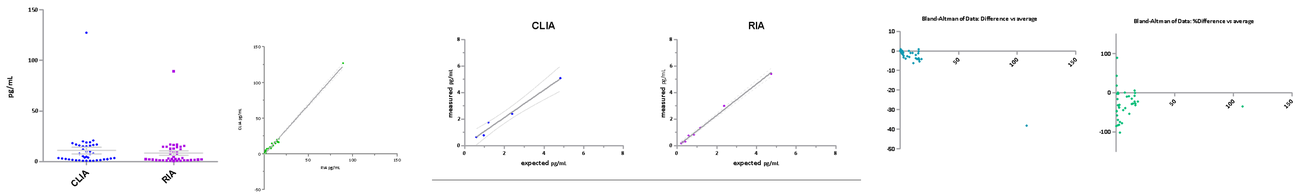
Usually, free Testosterone levels parallel the total testosterone levels. However, there are a number of conditions and medications that are known to increase or decrease the SHBG concentration, which may cause total testosterone concentration to change without necessarily influencing the free testosterone concentration. Free testosterone measurements are better indicators of mild hypogonadism in pubertal boys and adult men with mild decreases of total testosterone without LH abnormalities and in polycystic ovarian syndrome with low SHBG levels. We evaluated the analytical differences between two direct methods for the determination of free testosterone (competitive immunoluminometric assay CLIA; competitive radioimmunoassay RIA).

2 Methods

The analytical performance of quantitative determination of free Testosterone in human serum was evaluated with kit for CLIA on the Maglumi 600, SNIBE (Shenzhen New Industries Biomedical Engineering) fully autoanalyzer and with kit for RIA (Beckman Coulter Inc.). The within and between runprecision (CVA%) was determinated from the mean of four replicates for five separate run, each with two samples at different concentrations. For the comparison between CLIA and the RIA forty samples were measured. For the recovery test were analyzed two samples: A (pool of serum of healthy adult males) and B (pool of healthy adult females). Serial dilutions were performed; then the recovery of the measured expected concentrations (acceptability range 90-110%), the best-fit values and linear correlation coefficient (r^2) were calculate. For accuracy a Bland-Altman plot was performed.

3 Results

ANALITICAL VARIATION		RECOVERY						METHOD COMPARISON	
PRECISION			CLIA			RIA		BLAND ALTMAN	
	CVA %	sample dilutions	expected pg/mL	measured pg/mL	recovery %	expected pg/mL	measured pg/mL	recovery %	difference	
CLIA		A	9.65			9.50				
Pool1 (x=1,87 pg/mL**)**		1/2	4.83	5,07	105	4.75	5.40	113	Bias	-2.63
within run	7.6	1/4	2.41	2,38	99	2.38	2.98	125	SD of bias	6.04
between run	5.2	1/8	1.21	1,73	143	1.19	1.33	112	95% Limits of Agreement	
Pool2 (x=8.51 pg/mL**)**		1/16	0.60	0.62	103	0.59	0.74	125	From	-14.49
within run	3.82	1/32	0.30	< 0.50	-	0.30	0.25	84	To	9.21
between run	4.4	B	1.94		-	1.71				
		1/2	0.97	0.76	78	0.86	0.82	95		
RIA		1/4	0.49	< 0.50	-	0.43	0.29	68	percent difference	
Pool1 (x=1,96 pg/mL**)**		1/8	0.24	< 0.50	-	0.21	0.17	80		
within run	5.9	1/16	0.12	< 0.50	-	0.11	< 0.20	-	Bias	-29.46
between run	6.8	Best-fit values							SD of bias	36.22
pool2 (x=8.98 pg/mL**)**		Slope		1.038+/- 0.092			1.177 +/- 0.035		95% Limits of Agreement	
within run	4.5	Y-intercept		0.031 +/- 0.232			0.077 +/- 0.070		From	-100.47
between run	8.9	r^2		0.976			0.995		To	41.53



4 Conclusion

Our results show an acceptable assay performance: there are no analytical differences between two methods. Direct chemiluminescent immunoassay test kits are the easiest and fastest available methods of assessing free testosterone. However, both methods show relevant limitations to clinical use. The analytical sensitivity of both methods was insensitive for assessing low levels of free testosterone, such as may be found in females of any age or in less than 16 years old males. In these subjects the concentrations of free testosterone will continue to be reffered to a laboratory that uses a more accurate method (LC-MS/MS). The laboratory should provide the clinician with information about the sensitivity and specificity of the assay when using these methods.

5 References

Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab.2007; 92:405-13.