LABORATORY ALLIANCE of Central New York, LLC

Testosterone by LC/MS/MS and Associated Batteries: Free and Total Testosterone, LC/MS/MS Bioavailable Testosterone, LC/MS/MS

Effective September 17, 2013, Laboratory Alliance of Central New York will offer in-house testing for testosterone by liquid chromatography with tandem mass spectrometry detection (LC/MS/MS). This highly sophisticated methodology provides the sensitivity desired for evaluation of testosterone levels in females, children and hypogonadal males, with a low-end reporting limit of 2.5 ng/dL. In addition to the new test offering, we have also revised our testosterone evaluation batteries for assessment of free and bioavailable testosterones.

Testosterone in serum is largely protein-bound to either SHBG (sex hormone binding globulin) or weakly to albumin. A small fraction of testosterone in serum remains as the free hormone. Although testosterone is most notable for its androgenic properties, and its importance for male health, it is also important for female health. The primary function of testosterone in the male is as a sex hormone; it is responsible for the development of male sex organs and secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair.[1] The primary role of testosterone in females is as an estrogen precursor. In both males and females testosterone is important for health and well-being and plays an important role in bone metabolism, bone remodeling, and prevention of osteoporosis.[2,3]

The circulating concentration of testosterone in women is only 5-10% of that in men. In children, the circulating concentration of testosterone is highly age and sex dependent with reference ranges extending down to less than 5% of the adult male testosterone concentration.

The sensitive and specific measurement of circulating levels of testosterone in women is essential for the investigation of androgen disorders such as alopecia, acne, and hirsutism; and for the detection of androgen-secreting tumors.[4,5] In children, the measurement of circulating levels of testosterone is used for diagnosis, treatment, and gender assignment of newborns and young infants with ambiguous genitalia.[6] It is also used for pubertal stage determination and follow-up of children with precocious or delayed puberty.[7,8]

Automated immunoassays are widely used and are capable of accurately and precisely measuring the testosterone concentrations found in males. However, they lack the sensitivity and specificity to reliably measure the low testosterone concentrations found in women and children.[9,10] LC/MS/MS (liquid chromatography tandem mass spectrometry) testosterone methods have gained popularity in recent years because they can achieve the required sensitivity and specificity for the low concentrations typical of females and children.

As previously mentioned, testosterone circulates as free hormone (approximately 2%) or bound to plasma proteins (98%). Free testosterone is not routinely measured; rather the concentration is derived from a mathematical expression based on the constant for the binding of testosterone to sex hormone binding globulin (SHBG). For years it was believed that the free fraction of testosterone was the only biologically active fraction. However, it is now known that testosterone is weakly bound to albumin and readily dissociates in capillary beds, thereby becoming readily available for tissue uptake. All non-SHBG bound testosterone therefore should be considered bioavailable (free and albumin-bound), which in males represents about 35% of the total testosterone.

Total testosterone and general interpretation of testosterone abnormalities in **males**:

- Decreased testosterone levels indicate partial or complete hypogonadism. Serum testosterone levels are usually below the reference range.
- Increased testosterone levels in prepubertal boys are seen in precocious puberty. Further work-up is necessary to determine the cause(s) of precocious puberty.

Total testosterone and general interpretation of testosterone abnormalities in **females**:

- Decreased testosterone levels may be observed in primary or secondary ovarian failure, analogous to the situation in men, alongside the more prominent changes in female hormone levels. Most women with oophorectomy have a significant decrease in testosterone levels.
- Increased testosterone levels may be seen in:
    1. Congenital adrenal hyperplasia – nonclassical (mild) variants may not present in childhood but during or after puberty. In addition to testosterone, multiple other androgens or androgen precursors are elevated, such as 17OH-progesterone, often to a greater degree than testosterone.
    2. Prepubertal girls – analogous to males, but at lower levels, increased levels of testosterone are seen in precocious puberty.
    3. Ovarian or adrenal neoplasms – high estrogen values also may be observed, and LH and FSH are low or normal. Testosterone-producing ovarian or adrenal neoplasms often produce total testosterone values >200 ng/dL.
    4. Polycystic ovarian syndrome – hirsutism, acne, menstrual disturbances, insulin resistance and, frequently, obesity, form part of this syndrome. Total testosterone levels may be normal or mildly elevated and uncommonly >200 ng/dL.

The following table summarizes Laboratory Alliance's testosterone menu, incorporating both newly implemented LC/MS/MS-based testosterone testing and immunoassay-based testing batteries that were implemented earlier this year.

Test Name	Test Code	Includes	Suggested patient population
Testosterone	TSTR	Total testosterone by immunoassay (limit of detection 10 ng/dL)	Adult males, including boys 14 years and older.
Testosterone LC	TSTLC	Total testosterone by LCMS (limit of detection 2.5 ng/dL)	Females, children, hypogonadal males
Testosterone, Free and Total	TSTRF	Total testosterone by immunoassay, SHBG, calculated free testosterone and % free testosterone.	Adult males, including boys 14 years and older.
Testosterone LC, Free and Total	TSTLCF	Total testosterone by LCMS, SHBG and calculated free testosterone.	Females, children, hypogonadal males
Testosterone, Bioavailable	TSTRB	Total testosterone by immunoassay, SHBG, calculated bioavailable testosterone, free testosterone and % free testosterone.	Adult males, including boys 14 years and older.
Testosterone LC, Bioavailable	TSTLCB	Total testosterone by LCMS, SHBG, calculated bioavailable testosterone and free testosterone.	Females, children, hypogonadal males

Test details, specimen requirements and ordering information are included in the table below:

	Immunoassay Testosterone Batteries	LCMS Testosterone Batteries
Test Codes:		
Total Testosterone	TSTR	TSTLC
Total and Free Testosterone	TSTRF	TSTLCF
Total, Bioavailable and Free Testosterone	TSTRB	TSTLCB
Specimen requirements:	One 5 mL gold top tube (SST). 2 mL serum required. Plasma (heparin) is also acceptable.	One 5 mL gold top tube (SST). 2 mL serum required.
Storage and Transport:	Centrifuge within 2 hours of collection. Transport to laboratory refrigerated or ambient.	
Stability:	Refrigerated: 2 days	Refrigerated: 3 days
Unacceptable Conditions:		Plasma
Testing Schedule:	Daily	Set up Tuesdays and Fridays, reported on Wednesdays and Mondays
CPT Codes:		
Total Testosterone	84403	84403
Total and Free Testosterone	84403, 84270	84403, 84270
Total, Bioavailable and Free Testosterone	84403, 84270	84403, 84270
Billing Codes:		
Total Testosterone	1010097	1010457
Total and Free Testosterone	1010459	1010462
Total, Bioavailable and Free Testosterone	1010465	1010468

A complete set of age- and gender-specific reference intervals is available on our website at www.laboratoryalliance.com.

Questions regarding these tests may be directed to Cheryl Haskins, MS, MT(ASCP)SC, Manager, Chemistry and Referral Testing, at 315-410-7014 or cherylhaskins@lacny.com.

References:

1. Mooradian, AD, Morley, JE, Korenman, SG. Biological actions of androgens. Endocr Rev 1987; 8:1-28.
2. Bassil, N, Alkaade, S, Morley, JE. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag 2009; 5:427-48.
3. Tuck, SP, Francis, RM. Testosterone, bone and osteoporosis. Frontiers of Hormone Research 2009; 37:123-32.
4. Rosenfield, RL. Pilosebaceous physiology in relation to hirsutism and acne [Review]. Clin Endocrinol Metab 1986;15:341-62.
5. Waggoner, W, Boots, LR, Azziz, R. Total testosterone and DHEAS levels as predictors of androgen-secreting neoplasms: a populational study. Gynecol Endocrinol 1999;16:394-400.
6. Migeon, CJ, Berkovitz, GD, Brown, TR. Sexual differentiation and ambiguity. In: Kappy, MS, Blizzard, RM, Migeon, CJ, eds. The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield, IL: Charles C. Thomas, 1994:573-715.
7. Lee, PA. Laboratory monitoring of children with precocious puberty. Arch Pediatr Adolesc Med 1994; 148:369-76.
8. Kulin, HE, Finkelstein, JW, D'Arcangelo, MR, Susman, EJ, Chinchilli, V, Kunselman, S. Diversity of pubertal testosterone changes in boys: constitutional delay in growth and /or adolescence. J Pediatr Endocrinol Metab 1997; 10:395-400.
9. Wang, C, Catlin, DH, Demers, LM, Starcevic, B, Swerdloff, R. Measurement of total serum testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab 2004; 89:534-43.
10. Taieb, J, Mathian, B, Millot, F, Patricot, MC, Mathieu, E, Queyrel, N, Lacroix, I, Somma-Delpero, C, Boudou, P. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem. 2003; 49:1381-95.

8/22/13, cmh