Overview

Useful For

Second- or third-order test for evaluating testosterone status (eg, when abnormalities of sex hormone-binding globulin are present)

Profile Information

Test ID	Reporting Name	Available Separately	Always Performed
TTST	Testosterone, Total, S	Yes	Yes
FRTST	Testosterone, Free, S	No	Yes
BATS	Testosterone, Bioavailable, S	No	Yes

Method Name

FRTST: Equilibrium Dialysis

TTST: Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

BATS: Differential Precipitation

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Advisory Information

The preferred test for diagnosis of mild abnormalities of testosterone homeostasis, particularly if abnormalities in sex hormone-binding globulin (SHBG) function or levels are present, is TTBS / Testosterone, Total and Bioavailable, Serum.

Necessary Information

Patient's age and sex are required.

Specimen Required

Container/Tube: Red top (serum gel/SST are **not** acceptable)

Specimen Volume: 3.5 mL

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum Red	Refrigerated (preferred)	14 days	
	Frozen	60 days	

Clinical and Interpretive

Clinical Information

Testosterone is the major androgenic hormone. It is responsible for the development of the male external genitalia and secondary sexual characteristics. In females, its main role is as an estrogen precursor. In both genders, it also exerts anabolic effects and influences behavior.

In men, testosterone is secreted by the testicular Leydig cells and, to a minor extent, by the adrenal cortex. In premenopausal women, the ovaries are the main source of testosterone with minor contributions by the adrenals and peripheral tissues. After menopause, ovarian testosterone production is significantly diminished. Testosterone production in testes and ovaries is regulated via pituitary-gonadal feedback involving luteinizing hormone (LH) and, to a lesser degree, inhibins and activins.

Most circulating testosterone is bound to sex hormone-binding globulin (SHBG), which, in men, also is called testosterone-binding globulin. A lesser fraction is albumin bound and a small proportion exists as free hormone. Historically, only free testosterone was thought to be the biologically active component. However, testosterone is weakly bound to serum albumin and dissociates freely in the capillary bed, thereby becoming readily available for tissue uptake. All non-SHBG-bound testosterone is therefore considered bioavailable.

During childhood, excessive production of testosterone induces premature puberty in boys and masculinization in girls. In adult women, excess testosterone production results in varying degrees of virilization, including hirsutism, acne, oligo-amenorrhea, or infertility. Mild-to-moderate testosterone elevations are usually asymptomatic in males but can cause distressing symptoms in females. The exact causes for mild-to-moderate elevations in testosterone often remain obscure. Common causes of pronounced elevations of testosterone include genetic conditions (eg, congenital adrenal hyperplasia); adrenal, testicular, and ovarian tumors; and abuse of testosterone or gonadotrophins by athletes.

Decreased testosterone in females causes subtle symptoms. These may include some decline in libido and nonspecific mood changes. In males, it results in partial or complete degrees of hypogonadism. This is characterized by changes in male secondary sexual characteristics and reproductive function. The cause is either primary or secondary/tertiary (pituitary/hypothalamic) testicular failure. In adult men, there also is a gradual modest, but progressive, decline in testosterone production starting between the fourth and sixth decades of life. Since this is associated with a simultaneous increase of SHBG levels, bioavailable testosterone may decline more significantly than apparent total testosterone, causing nonspecific symptoms similar to those observed in testosterone deficient females. However, severe hypogonadism, consequent to aging alone, is rare.

Measurement of total testosterone (TTST / Testosterone, Total, Mass Spectrometry, Serum) is often sufficient for diagnosis, particularly if it is combined with measurements of LH and follicle-stimulation hormone (FSH) (LH / Luteinizing Hormone [LH], Serum and FSH / Follicle-Stimulating Hormone [FSH], Serum). However, these tests may be insufficient for diagnosis of mild abnormalities of testosterone homeostasis, particularly if abnormalities in SHBG (SHBG / Sex Hormone-Binding Globulin [SHBG], Serum) function or levels are present. Additional measurements of free testosterone or bioavailable testosterone are recommended in this situation; bioavailable testosterone (see TTBS / Testosterone, Total and Bioavailable, Serum) is the preferred assay.

Reference Values

TESTOSTERONE, TOTAL

Males

0-5 months: 75-400 ng/dL

6 months-9 years: <7-20 ng/dL

10-11 years: <7-130 ng/dL

12-13 years: <7-800 ng/dL

14 years: <7-1,200 ng/dL

15-16 years: 100-1,200 ng/dL

17-18 years: 300-1,200 ng/dL

> or =19 years: 240-950 ng/Dl

Tanner Stages*

I (prepubertal): <7-20

II: 8-66

III: 26-800

IV: 85-1,200

V (young adult): 300-950

Females

0-5 months: 20-80 ng/dL

6 months-9 years: <7-20 ng/dL

10-11 years: <7-44 ng/dL

12-16 years: <7-75 ng/dL

17-18 years: 20-75 ng/dL

> or =19 years: 8-60 ng/dL

Tanner Stages*

I (prepubertal): <7-20

II: <7-47

III: 17-75

IV: 20-75

V (young adult): 12-60

*Puberty onset (transition from Tanner stage I to Tanner stage II) occurs for boys at a median age of 11.5 (+/-2) years and for girls at a median age of 10.5 (+/-2) years. There is evidence that it may occur up to 1 year earlier in obese girls and in African American girls. For boys, there is no definite proven relationship between puberty onset and body weight or ethnic origin. Progression through Tanner stages is variable. Tanner stage V (young adult) should be reached by age 18.

TESTOSTERONE, FREE

Males (adult):

20-<25 years: 5.25-20.7 ng/dL

25-<30 years: 5.05-19.8 ng/dL

30-<35 years: 4.85-19.0 ng/dL

35-<40 years: 4.65-18.1 ng/dL

40-<45 years: 4.46-17.1 ng/dL

45-<50 years: 4.26-16.4 ng/dL

50-<55 years: 4.06-15.6 ng/dL

55-<60 years: 3.87-14.7 ng/dL

60-<65 years: 3.67-13.9 ng/dL

65-<70 years: 3.47-13.0 ng/dL

70-<75 years: 3.28-12.2 ng/dL

75-<80 years: 3.08-11.3 ng/dL

80-<85 years: 2.88-10.5 ng/dL

85-<90 years: 2.69-9.61 ng/dL

90-<95 years: 2.49-8.76 ng/dL

95-100+ years: 2.29-7.91 ng/dL

Males (children):

<1 year: Term infants

1-15 days: 0.20-3.10 ng/dL*

16 days-1 year: Values decrease gradually from newborn (0.20-3.10 ng/dL) to prepubertal levels

*Citation: J Clin Endocrinol Metab 1973;36(6):1132-1142

1-8 years: <0.04-0.11 ng/dL

9 years: <0.04-0.45 ng/dL

10 years: <0.04-1.26 ng/dL

11 years: <0.04-5.52 ng/dL

12 years: <0.04-9.28 ng/dL

13 years: <0.04-12.6 ng/dL

14 years: 0.48-15.3 ng/dL

15 years: 1.62-17.7 ng/dL

16 years: 2.93-19.5 ng/dL

17 years: 4.28-20.9 ng/dL

18 years: 5.40-21.8 ng/dL

19 years: 5.36-21.2 ng/dL

Females (adult):

20-<25 years: 0.06-1.08 ng/dL

25-<30 years: 0.06-1.06 ng/dL

30-<35 years: 0.06-1.03 ng/dL

35-<40 years: 0.06-1.00 ng/dL

40-<45 years: 0.06-0.98 ng/dL

45-<50 years: 0.06-0.95 ng/dL

50-<55 years: 0.06-0.92 ng/dL

55-<60 years: 0.06-0.90 ng/dL

60-<65 years: 0.06-0.87 ng/dL

65-<70 years: 0.06-0.84 ng/dL

70-<75 years: 0.06-0.82 ng/dL

75-<80 years: 0.06-0.79 ng/dL

80-<85 years: 0.06-0.76 ng/dL

85-<90 years: 0.06-0.73 ng/dL

90-<95 years: 0.06-0.71 ng/dL

95-100+ years: 0.06-0.68 ng/dL

Females (children):

<1 year: Term infants

1-15 days: 0.06-0.25 ng/dL*

16 days-1 year: Values decrease gradually from newborn (0.06-0.25 ng/dL) to prepubertal levels

*Citation: J Clin Endocrinol Metab 1973;36(6):1132-1142

1-4 years: <0.04 ng/dL

5 years: <0.04-0.07 ng/dL

6 years: <0.04-0.14 ng/dL

7 years: <0.04-0.23 ng/dL

8 years: <0.04-0.34 ng/dL

9 years: <0.04-0.46 ng/dL

10 years: <0.04-0.59 ng/dL

11 years: <0.04-0.72 ng/dL

12 years: <0.04-0.84 ng/dL

13 years: <0.04-0.96 ng/dL

14 years: <0.04-1.06 ng/dL

15-18 years: <0.04-1.09 ng/dL

19 years: 0.06-1.08 ng/dL

TESTOSTERONE, BIOAVAILABLE

Males

< or =19 years: not established

20-29 years: 83-257 ng/dL

30-39 years: 72-235 ng/dL

40-49 years: 61-213 ng/dL

50-59 years: 50-190 ng/dL

60-69 years: 40-168 ng/dL

> or =70 years: not established

Females (non-oophorectomized)

< or =19 years: not established

20-50 years (on oral estrogen): 0.8-4.0 ng/dL

20-50 years (not on oral estrogen): 0.8-10 ng/dL

>50 Years: not established

Interpretation

Total testosterone and general interpretation of testosterone abnormalities:

In male patients:

Decreased testosterone levels indicate partial or complete hypogonadism. Serum testosterone levels are usually below the reference range. The cause is either primary or secondary/tertiary (pituitary/hypothalamic) testicular failure.

Primary testicular failure is associated with increased luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels, and decreased total, bioavailable, and free testosterone levels. Causes include:

-Genetic causes (eg, Klinefelter syndrome, XXY males)

-Developmental causes (eg, testicular maldescent)

-Testicular trauma or ischemia (eg, testicular torsion, surgical mishap during hernia operations)

-Infections (eg, mumps)

-Autoimmune diseases (eg, autoimmune polyglandular endocrine failure)

-Metabolic disorders (eg, hemochromatosis, liver failure)

-Orchidectomy

Secondary/tertiary hypogonadism, also known as hypogonadotrophic hypogonadism, shows low testosterone and low, or inappropriately "normal," LH/FSH levels; causes include:

-Inherited or developmental disorders of hypothalamus and pituitary (eg, Kallmann syndrome, congenital hypopituitarism)

-Pituitary or hypothalamic tumors

-Hyperprolactinemia of any cause

-Malnutrition or excessive exercise

-Cranial irradiation

-Head trauma

-Medical or recreational drugs (eg, estrogens, gonadotropin releasing hormone [GnRH] analogs, cannabis)

Increased testosterone levels:

-In prepubertal boys, increased levels of testosterone are seen in precocious puberty. Further workup is necessary to determine the cause of precocious puberty

-In adult men, testicular or adrenal tumors or androgen abuse might be suspected if testosterone levels exceed the upper limit of the normal range by more than 50%.

Monitoring of testosterone replacement therapy:

Aim of treatment is normalization of serum testosterone and LH. During treatment with depot-testosterone preparations, trough levels of serum testosterone should still be within the normal range, while peak levels should not be significantly above the normal young adult range.

Monitoring of antiandrogen therapy:

Aim is usually to suppress testosterone levels to castrate levels or below (no more than 25% of the lower reference range value).

In female patients:

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:

-Congenital adrenal hyperplasia: non-classical (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 17 hydroxyprogesterone (OHPG / 17-Hydroxyprogesterone, Serum), often to a greater degree than testosterone.

-Prepubertal girls: analogous to males, but at lower levels, increased levels of testosterone are seen in precocious puberty.

-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 greater than 200 ng/dL.

-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, greater than 200 ng/dL.

Monitoring of testosterone replacement therapy:

The efficacy of testosterone replacement in females is under study. If it is used, total testosterone levels should be kept within the normal female range at all times. Bioavailable or free testosterone levels also should be monitored to avoid overtreatment.

Monitoring of antiandrogen therapy:

Antiandrogen therapy is most commonly employed in the management of mild-to-moderate "idiopathic" female hyperandrogenism, as seen in polycystic ovarian syndrome. Total testosterone levels are a relatively crude guideline for therapy and can be misleading. Therefore, bioavailable or free testosterone also should be monitored to ensure treatment adequacy. However, there are no universally agreed biochemical endpoints and the primary treatment end point is the clinical response.

Bioavailable and Free Testosterone:

Usually, bioavailable and free testosterone levels parallel the total testosterone levels. However, a number of conditions and medications are known to increase or decrease the SHBG (SHBG / Sex Hormone-Binding Globulin [SHBG], Serum) concentration, which may cause total testosterone concentration to change without necessarily influencing the bioavailable or free testosterone concentration, or vice versa:

-Treatment with corticosteroids and sex steroids (particularly oral conjugated estrogen) can result in changes in SHBG levels and availability of sex-steroid binding sites on SHBG. This may make diagnosis of subtle testosterone abnormalities difficult.

-Inherited abnormalities in SHBG binding.

-Liver disease and severe systemic illness.

-In pubertal boys and adult men, mild decreases of total testosterone without LH abnormalities can be associated with delayed puberty or mild hypogonadism. In this case, either bioavailable or free testosterone measurements are better indicators of mild hypogonadism than determination of total testosterone levels.

-In polycystic ovarian syndrome and related conditions, there is often significant insulin resistance, which is associated with low SHBG levels. Consequently, bioavailable or free testosterone levels may be more significantly elevated.

Either bioavailable or free testosterone should be used as supplemental tests to total testosterone in the above

situations. The correlation coefficient between bioavailable and free testosterone (by equilibrium dialysis) is 0.9606. However, bioavailable testosterone is usually the preferred test, as it more closely reflects total bioactive testosterone, particularly in older men. Older men not only have elevated SHBG levels, but albumin levels also may vary due to coexisting illnesses.

Cautions

Early morning testosterone levels in young male individuals are, on average, 50% higher than p.m. levels. Reference values were established using specimens collected in the morning.

Testosterone levels can fluctuate substantially between different days, and sometimes even more rapidly. Assessment of androgen status should be based on more than a single measurement.

The low end of the normal reference range for total testosterone in prepubertal subjects is not yet established.

While free testosterone can be used for the same indications as bioavailable testosterone, determination of bioavailable testosterone levels may be superior to free testosterone measurement in most situations.

Clinical Reference

1. Manni A, Pardridge WM, Cefalu W, et al: Bioavailability of albumin-bound testosterone. J Clin Endocrinol Metab. 1985;61:705

2. New MI, Josso N: Disorders of gonadal differentiation and congenital adrenal hyperplasia. Endocrinol Metab Clin North Am. 1988;17:339-366

3. Dumesic DA: Hyperandrogenic anovulation: a new view of polycystic ovary syndrome. Postgrad Obstet Gynecol. 1995 June;15(13)

4. Morley JE, Perry HM 3rd: Androgen deficiency in aging men: role of testosterone replacement therapy. J Lab Clin Med. 2000;135:370-378

5. Goldman AL, Bhasin S, Wu FCW, et al: A reappraisal of testosterone's binding in circulation: physiological and clinical implications. Endocr Rev. 2017;38(4):302-324. doi: 10.1210/er.2017-00025

Performance

Method Description

Total Testosterone:

Deuterated stable isotope (d3-testosterone) is added to a 0.2-mL serum sample as an internal standard. Protein is precipitated from the mixture by the addition of acetonitrile. The testosterone and internal standard are extracted from the resulting supernatant by an online extraction utilizing high-throughput liquid chromatography (HTLC). This is followed by conventional liquid chromatography and analysis on a tandem mass spectrometer equipped with a heated nebulizer ion source.(Wang C, Catlin DH, Demers LM, et al: Measurement of total testosterone in adult men: comparison of current laboratory methods versus liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab. 2004;89:534-543; Taieb J, Mathian B, Millot F, et al: Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem. 2003;49:1381-1395)

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Free Testosterone:

This method is based on equilibrium dialysis in which free-labeled testosterone is allowed to pass through a semipermeable membrane, whereas testosterone bound to the sex hormone-binding globulin (SHBG) remains inside the dialysis tubing. After dialysis, radioactivity is measured both inside and outside the tubing; the free testosterone results are expressed as a percentage of total testosterone. The resulting percentage is multiplied by the total testosterone concentration obtained by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and an absolute free testosterone value is obtained.(Vermeulen A, Stoica T, Verdonck L: The apparent free testosterone concentration, an index of androgenicity. J Clin Endocrinol Metab. 1971;33:759-767; Bammann BL, Coulam C, Jiang NS: Total and free testosterone during pregnancy. Am J Obstet Gynecol. 1980;137:293-298)

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Bioavailable Testosterone:

The method is based on the differential precipitation of SHBG by ammonium sulfate following equilibration of the serum specimen and tracer amounts of tritium-labeled testosterone. The results are expressed as the percent of testosterone free or albumin bound (not precipitated with SHBG) compared to an albumin standard. The product of this percentage and the total testosterone measurement is the total bioavailable testosterone.(Wheeler MJ: The determination of bioavailable testosterone. Ann Clin Biochem. 1995;32:345-357)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Saturday; 1 p.m.

Analytic Time

3 days

Maximum Laboratory Time

5 days

Specimen Retention Time

2 weeks

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

84402

84403

84410

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
TTFB	Testosterone, Total, Bio, Free, S	58716-2

Result ID	Test Result Name	Result LOINC Value
82978	Testosterone, Bioavailable, S	2990-0
3631	Testosterone, Free, S	2991-8
8533	Testosterone, Total, S	2986-8