



Test Definition: TTFB

Testosterone, Total, Bioavailable, and Free,
Serum

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

BATS: Differential Precipitation/Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

FRTST: Equilibrium Dialysis/Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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

NY State Available

Yes

Specimen

Specimen Type

Serum Red

Ordering Guidance

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

Necessary Information

Patient's age and sex are required.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)

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

Submission Container/Tube: Plastic vial

Specimen Volume: 3.5 mL

Collection Instructions: Centrifuge and aliquot serum into a plastic vial

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	Reject
Gross icterus	OK

Specimen Stability Information

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

Clinical & 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 female patients, its main role is as an estrogen precursor. In both sexes, 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 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 male patients but can cause distressing symptoms in female patients. 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 female patients causes subtle symptoms. These may include some decline in libido and nonspecific mood changes. In male patients, 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 men, there also is a gradual, modest but progressive decline in testosterone production starting between the 4th and 6th 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 women. 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 (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 (SHBG1 / Sex Hormone-Binding Globulin, 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

*Forest MG, Cathiard AM, Bertrand JA. Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: use of a sensitive radioimmunoassay for testosterone. J Clin Endocrinol Metab. 1973;36(6):1132-1142

1-8 years: <0.13 ng/dL

9 years: <0.13-0.45 ng/dL

10 years: <0.13-1.26 ng/dL

11 years: <0.13-5.52 ng/dL

12 years: <0.13-9.28 ng/dL

13 years: <0.13-12.6 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: <0.13-1.08 ng/dL
25-<30 years: <0.13-1.06 ng/dL
30-<35 years: <0.13-1.03 ng/dL
35-<40 years: <0.13-1.00 ng/dL
40-<45 years: <0.13-0.98 ng/dL
45-<50 years: <0.13-0.95 ng/dL
50-<55 years: <0.13-0.92 ng/dL
55-<60 years: <0.13-0.90 ng/dL
60-<65 years: <0.13-0.87 ng/dL
65-<70 years: <0.13-0.84 ng/dL
70-<75 years: <0.13-0.82 ng/dL
75-<80 years: <0.13-0.79 ng/dL
80-<85 years: <0.13-0.76 ng/dL
85-<90 years: <0.13-0.73 ng/dL
90-<95 years: <0.13-0.71 ng/dL
95-100+ years: <0.13-0.68 ng/dL

Females (children):

<1 year: Term infants

1-15 days: <0.13-0.25 ng/dL*

16 days-1 year: Values decrease gradually from newborn (<0.13-0.25 ng/dL) to prepubertal levels

*Forest MG, Cathiard AM, Bertrand JA. Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: use of a sensitive radioimmunoassay for testosterone. J Clin Endocrinol Metab. 1973;36(6):1132-1142

1-4 years: <0.13 ng/dL
5 years: <0.13 ng/dL
6 years: <0.14 ng/dL
7 years: <0.13-0.23 ng/dL
8 years: <0.13-0.34 ng/dL
9 years: <0.13-0.46 ng/dL
10 years: <0.13-0.59 ng/dL
11 years: <0.13-0.72 ng/dL
12 years: <0.13-0.84 ng/dL
13 years: <0.13-0.96 ng/dL
14 years: <0.13-1.06 ng/dL

15-18 years: <0.13-1.09 ng/dL

19 years: <0.13-1.08 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): 0.80-4.0 ng/dL

20-50 years (not on oral estrogen): 0.80-10 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 hypogonadotropic 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 testosterone replacement therapy:

Aim of treatment is normalization of serum testosterone and LH. When undergoing testosterone replacement therapy 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 antiandrogen therapy:

Aim is usually to suppress testosterone levels to castrate levels or below. Evidence shows that lowering testosterone levels to less than 20 ng/dL improves patient survival and delays disease progression.

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 boys, 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 above 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 exceed 200 ng/dL.

Monitoring testosterone replacement therapy:

The only evidence-based indication for testosterone for women is for hypoactive sexual desire disorder/dysfunction.

There are insufficient data for using testosterone for any other symptom/condition or for disease prevention. If it is used, total testosterone levels should always be kept within the normal range for females. Bioavailable or free testosterone levels also should be monitored to avoid overtreatment.

Monitoring antiandrogen therapy:

Antiandrogen therapy is most frequently 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 end points 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 sex hormone-binding globulin (SHBG) 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 have elevated SHBG levels, but albumin levels may also 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.

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.

Oral contraceptives have been shown to reduce levels of testosterone and increase levels of SHBG. Therefore, levels of free and bioavailable testosterone are coincidentally decreased.

Clinical Reference

1. Manni A, Pardridge WM, Cefalu W, et al. Bioavailability of albumin-bound testosterone. *J Clin Endocrinol Metab.* 1985;61(4):705-710
2. New MI, Josso N. Disorders of gonadal differentiation and congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am.* 1988;17(2):339-366
3. Dumesic DA. Hyperandrogenic Anovulation: A New View of Polycystic Ovary Syndrome. *Postgraduate Obstetrics and*

Gynecology. Vol 15, No.13, June 1995

4. Morley JE, Perry HM 3rd. Androgen deficiency in aging men: role of testosterone replacement therapy. *J Lab Clin Med.* 2000;135(5):370-378. doi:10.1067/mlc.2000.106455
5. Sizonenko PC, Paunier L. Hormonal changes in puberty III: Correlation of plasma dehydroepiandrosterone, testosterone, FSH, and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche. *J Clin Endocrinol Metab.* 1975;41(5):894-904. doi:10.1210/jcem-41-5-894
6. Goudas VT, Dumesic DA. Polycystic ovary syndrome. *Endocrinol Metab Clin North Am.* 1997;26(4):893-912. doi:10.1016/s0889-8529(05)70286-3
7. Braunstein GD. Androgen insufficiency in women: summary of critical issues. *Fertil Steril.* 2002;77 Suppl 4:S94-S99. doi:10.1016/s0015-0282(02)02962-x
8. Juul A, Skakkebaek NE. Androgens and the ageing male. *Hum Reprod Update.* 2002;8(5):423-433. doi:10.1093/humupd/8.5.423
9. Hackbarth JS, Hoyne JB, Grebe SK, Singh RJ. Accuracy of calculated free testosterone differs between equations and depends on gender and SHBG concentration. *Steroids.* 2011;76(1-2):48-55. doi:10.1016/j.steroids.2010.08.008
10. Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. 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
11. Mulhall JP, Trost LW, Brannigan RE, et al. Evaluation and Management of Testosterone Deficiency: AUA Guideline. *J Urol.* 2018;200(2):423-432
12. Trost L. Update to the Testosterone Guideline. *J Urol.* 2024;211(4):608-610
13. Al-Sharefi A, Quinton R. Current National and International Guidelines for the Management of Male Hypogonadism: Helping Clinicians to Navigate Variation in Diagnostic Criteria and Treatment Recommendations. *Endocrinol Metab (Seoul).* 2020;35(3):526-540. doi:10.3803/EnM.2020.760
14. Davis SR, Baber R, Panay N, et al. Global Consensus Position Statement on the Use of Testosterone Therapy for Women. *J Clin Endocrinol Metab.* 2019;104(10):4660-4666. doi:10.1210/jc.2019-01603
15. Martin KA, Anderson RR, Chang RJ, et al. Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2018;103(4):1233-1257. doi:10.1210/jc.2018-00241
16. Crawford ED, Heidenreich A, Lawrentschuk N, et al. Androgen-targeted therapy in men with prostate cancer: evolving practice and future considerations. *Prostate Cancer Prostatic Dis.* 2019;22(1):24-38

Performance

Method Description

Testosterone, Total:

An internal standard (IS) of isotopically carbon-13 labeled testosterone is added to the serum sample. Protein is precipitated from the mixture by the addition of a crash solution followed by derivatization of the testosterone and IS using hydroxylamine. The derivatized testosterone and internal standard are extracted from the resulting supernatant by an online extraction utilizing high-throughput liquid chromatography. This is followed by conventional liquid chromatography and analysis on a tandem mass spectrometer equipped with an electrospray ion source. Epitestosterone does not interfere with this liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for total testosterone. (Unpublished Mayo method)

Testosterone, Free:

This method utilizes equilibrium dialysis to directly measure free testosterone. Patient sample is placed inside a dialysis well which is immersed in dialysis buffer. The sample is dialyzed for 23 hours on a rotator at 37 degrees C. During buffered dialysis, any testosterone which is unbound to sex hormone binding globulin (SHBG) or albumin is free to pass through the semi-permeable dialysis membrane while those testosterone molecules bound to the binding proteins will be held inside the membrane. After dialysis, the buffered dialysate is analyzed for free testosterone by LC-MS/MS with isotopically labeled IS after derivatization.(Unpublished Mayo method)

Testosterone, Bioavailable:

The method is based on the differential precipitation of sex hormone binding globulin (SHBG) by ammonium sulfate. SHBG bound testosterone is precipitated in patient sample using saturated ammonium sulfate, leaving the bioavailable testosterone in the supernatant. After addition of carbon-13 labeled testosterone internal standard (IS), the bioavailable testosterone and IS are extracted from the supernatant using liquid-liquid extraction. The bioavailable testosterone and IS are then derivatized and analyzed by LC-MS/MS. Bioavailable testosterone shows a strong correlation to free testosterone by equilibrium dialysis and is considered the preferred test in many cases.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Saturday

Report Available

3 to 8 days

Specimen Retention Time

2 weeks

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Fees & 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 account representative. 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. It has not been cleared or approved by the US 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	2991-8
8533	Testosterone, Total, S	2986-8