



Test Definition: TTST

Testosterone, Total, Mass Spectrometry,
Serum

Overview

Useful For

Evaluating men with symptoms or signs of possible hypogonadism, such as loss of libido, erectile dysfunction, gynecomastia, osteoporosis, or infertility

Evaluating boys with delayed or precocious puberty

Monitoring testosterone replacement therapy

Monitoring antiandrogen therapy (eg, used in prostate cancer, precocious puberty, treatment of idiopathic hirsutism, male-to-female transgender disorders, etc.)

Evaluating women with hirsutism, virilization, and oligoamenorrhea

Evaluating women with symptoms or signs of possible testosterone deficiency

Evaluating infants with ambiguous genitalia or virilization

Diagnosing androgen-secreting tumors

Testing Algorithm

For information see [Steroid Pathways](#)

Special Instructions

- [Steroid Pathways](#)

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Serum Red

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:** 1 mL**Collection Instructions:** Centrifuge and aliquot serum into a plastic vial.**Specimen Minimum Volume**

0.215 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	OK
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 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 adrenal glands 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, is also 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, oligomenorrhea, or infertility. Mild-to-moderate testosterone elevations are usually asymptomatic in male patients but can cause distressing symptoms in female patients. The exact cause for mild-to-moderate elevations of testosterone often remains obscure. Common causes of pronounced elevations 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 fourth and sixth decade 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 is often sufficient for diagnosis, particularly if it is combined with measurements of LH and follicle-stimulating 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 bioavailable (TTBS / Testosterone, Total and Bioavailable, Serum) or free testosterone (TGRP / Testosterone Total and Free, Serum) are recommended in this situation.

For more information see [Steroid Pathways](#)

Reference Values

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.

Interpretation

In male patients:

Decreased testosterone levels indicate partial or complete hypogonadism. In 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
- 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 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, such as 17-hydroxyprogesterone (OHPG / 17-Hydroxyprogesterone, Serum), are elevated, 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, then levels should be kept within the normal range for females at all times. Bioavailable or free testosterone levels should also be monitored to avoid overtreatment.

Monitoring 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 should also be monitored to ensure treatment adequacy; see TTFB / Testosterone, Total, Bioavailable, and Free, Serum. However, there are no universally

agreed biochemical end points, and the primary treatment end point is the clinical response.

Cautions

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

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

Oral contraceptives have been shown to reduce levels of testosterone and increase levels of sex hormone-binding globulin. Therefore, levels of free and bioavailable testosterone are coincidentally decreased.

Supportive Data

While interferences, cross-reactivity, and lack of result comparability between different assays are a challenge for testosterone immunoassays, particularly at low testosterone concentrations, this method is based on liquid chromatography tandem mass spectrometry and provides reproducible and highly accurate testosterone measurements throughout the analytical range. Therefore, results will be lower than, and not directly comparable with, results obtained by immunoassays. Most immunoassays overestimate the true testosterone concentration by 10% to 300%, depending on the assay used and whether the measured concentration falls into the low, medium, or high range.

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. *Postgrad Obstet Gynecol.* 1995;15(13)
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
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
6. Goudas VT, Dumesic DA: Polycystic ovary syndrome. *Endocrinol Metab Clin North Am* 1997;26(4):893-912
7. Braunstein GD: Androgen insufficiency in women: summary of critical issues. *Fertil Steril.* 2002 Apr;77 Suppl 4:S94-99
8. Juul A, Skakkebaek NE: Androgens and the aging male. *Hum Reprod Update* 2002;8(5):423-433
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
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. doi:10.1016/j.juro.2018.03.115
12. Trost L. Update to the Testosterone Guideline. *J Urol.* 2024;211(4):608-610. doi:10.1097/JU.0000000000003855
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

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14. 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
15. 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. doi:10.1038/s41391-018-0079-0

Performance

Method Description

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 method for total testosterone.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Saturday

Report Available

2 to 4 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

84403

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
TTST	Testosterone, Total, S	2986-8

Result ID	Test Result Name	Result LOINC® Value
8533	Testosterone, Total, S	2986-8