



Testosterone, LC/MS/MS

Total: test code 15983X

Free and Total: test code 36170X

Free, Bioavailable, and Total: test code 14966X

Clinical Use

- Diagnose and monitor hyperandrogenic disorders such as polycystic ovary syndrome in women with alopecia, acne, and hirsutism
- Diagnose and monitor therapy in patients with androgen secreting neoplasms and congenital or non-classical (late-onset) adrenal hyperplasia
- Determine androgen status in children with precocious or delayed puberty, ambiguous genitalia, or unexplained virilization
- Diagnose testosterone deficiency in men
- Monitor prostate cancer therapies (gonadotropin-releasing hormone analogs and antiandrogens)

Clinical Background

Testosterone is produced by the testicular Leydig cells in males and by the adrenal glands (25%), ovaries (25%), and peripheral conversion of circulating androstenedione (50%) in females.¹ In both males and females, the majority of circulating testosterone is protein bound. Sex hormone binding globulin (SHBG), the major binding protein, binds 60% to 70% of the testosterone in circulation. The remaining testosterone circulates weakly bound to other proteins, primarily albumin, and as free (not bound to protein) testosterone. Albumin-bound testosterone accounts for 30% to 40% of the testosterone in circulation, and free, approximately 2%.

Testosterone bound to SHBG is biologically inactive because of the strong affinity between SHBG and testosterone. Free testosterone is biologically active, as is albumin bound (due to weak albumin-testosterone binding). Albumin-bound and free testosterone, together, are frequently referred to as the biologically active or bioavailable fraction. In most situations, the bioavailable fraction increases as total testosterone increases or as SHBG decreases.

In utero, testosterone is necessary for the development of male genitalia in 46,XY fetuses.² After birth, the serum concentration in boys remains approximately twice that of girls until puberty. In boys, a more than 10-fold increase during puberty leads to the development of

secondary sexual characteristics, whereas in girls, a 2-fold increase leads to the development of pubic and axillary hair.³ In women, serum testosterone concentration is approximately 5%-10% of that in men and is thought to be important in the maintenance of bone mineral density, mood, and libido.^{1,4} In men, testosterone is necessary for the maintenance of spermatogenesis, secondary sexual characteristics, bone density, muscle mass, and libido and is thought to play a role in memory recall.⁵

Evidence suggests traditional immunoassays are unable to accurately quantitate the low serum testosterone concentrations found in women and children,^{6,7} in men with androgen deficiencies,⁸ and in patients undergoing antiandrogenic therapies.⁸ Liquid chromatography tandem mass spectrometry (LC/MS/MS) has emerged as the method of choice for measuring testosterone in these populations because of markedly increased sensitivity and specificity.^{6,8} Additionally, turbulent flow LC/MS/MS, as used in this assay, requires lower sample volume and provides greater sensitivity than liquid/liquid or derivatization LC/MS/MS.⁹

Individuals Suitable for Testing

- Women and children with suspected androgen excess
- Newborns with ambiguous genitalia
- Children with evidence of precocious or delayed puberty
- Men with suspected testosterone deficiency
- Men with prostate cancer treated with gonadotropin-releasing hormone analogs and antiandrogen therapies

Specimen Requirements

Refrigerated serum (no-additive red-top tube) is preferred. Heparinized plasma (green-top tube) is acceptable. Serum collected in serum separator tubes (SST) is unacceptable.

- Total Testosterone: 0.5 mL; 0.18 mL minimum
- Free and Total Testosterone: 0.9 mL; 0.38 mL minimum
- Free, Bioavailable, and Total Testosterone: 2.8 mL; 1.3 mL minimum

Method

- Total Testosterone
 - Turbulent flow liquid chromatography tandem mass spectrometry (LC/MS/MS)
 - Analytical sensitivity: 1.0 ng/dL
 - Analytical specificity: no cross-reactivity with 30 testosterone-related steroid compounds
 - Reportable range: 1.0 ng/dL to 2000 ng/dL
 - CPT code*: 84403
- Free and Total Testosterone
 - Total: LC/MS/MS
 - Percent free: equilibrium dialysis
 - Free: calculated based on total and percent free
 - Aliases: testosterone index, dialyzable testosterone
 - CPT codes*: 84403, 84402
- Free, Bioavailable, and Total Testosterone
 - Total: LC/MS/MS
 - Free: calculated based on constants for the binding of testosterone to SHBG and albumin
 - Bioavailable: calculated based on constants for the binding of testosterone to SHBG and albumin
 - SHBG: immunochemiluminometric assay (ICMA)
 - Albumin: spectrophotometry
 - Alias: free, weakly bound, and total testosterone
 - CPT codes*: 84403, 84270, 82040

Reference Range

See Tables 1–3.

Interpretive Information

Testosterone is elevated in infants with congenital adrenal hyperplasia secondary to 21-hydroxylase or 11-hydroxylase deficiencies, conditions that cause masculinization of the genitalia in female fetuses.² Serum testosterone concentrations may also be increased or decreased in other disorders associated with ambiguous genitalia in newborns (Table 4).^{12,13} In adolescent children, elevated testosterone may be diagnostic of precocious puberty, whereas a decreased concentration may be indicative of hypogonadism in boys.³

In women, elevated serum testosterone commonly manifests as alopecia, severe acne, hirsutism, and/or menstrual disturbances. Elevations can result from androgen-secreting tumors of the adrenal gland or ovary, polycystic ovary syndrome, late onset congenital adrenal hyperplasia, or Cushing's syndrome.¹³

In men, decreased testosterone levels may be due to primary testicular failure (associated with elevated LH and FSH), secondary hypogonadism (associated with decreased LH and FSH), or treatment of prostate cancer with gonadotropin releasing hormone analogs or antiandrogens.¹⁴ Elevated testosterone levels may result from androgen-secreting tumors of the adrenal gland, late onset congenital adrenal hyperplasia, or Cushing's syndrome.⁵

Medical conditions altering serum concentrations of SHBG or albumin (eg, obesity or cirrhosis) may affect the total testosterone level, though free and bioavailable testosterone may remain normal. Additionally, certain hirsute females may have a normal total testosterone level while their free and bioavailable testosterone are elevated. Testosterone results should be interpreted in conjunction with other laboratory and clinical findings.

Table 1. Testosterone Reference Ranges in Adults

Age (years)	Total (LC/MS/MS) ^a (ng/dL)	Free and Total ^b		Free, Bioavailable, and Total ^c	
		% Free (percent)	Free (pg/mL)	Free (pg/mL)	Bioavailable (ng/dL)
Females					
18-69	2-45		0.1-6.4	0.2-5.0	0.5-8.5
70-94	2-40				
70-89			0.2-3.7	0.3-5.0	0.5-8.8
18-89		0.5-2.0			
Males					
18-69	250-1100		35-155	46-224	110-575
70-89	90-890		30-135	6.0-73	15-150
18-89		1.5-2.2			

^a Test code: 15983X, 36170X, 14966X.

^b Test code: 36170X.

^c Test code: 14966X.

Table 2. Testosterone Reference Ranges in Children and Adolescents

Age	Total (LC/MS/MS) ^a ng/dL	Free and Total ^b		Free, Bioavailable, and Total ^c	
		% Free	Free, pg/mL	Free, pg/mL	Bioavailable, ng/dL
Females					
Cord blood ^{10,11}	16-44				
1-10 d ^{10,11}	≤24				
1-3 mo ^{10,11}	≤17				
3-5 mo ^{10,11}	≤12				
5-7 mo ^{10,11}	≤13				
7-12 mo ^{10,11}	≤11				
1-5.9 y	≤8				
6-7.9 y	≤20				
5-9.9 y		0.28-1.81	0.2-5.0		
1-10.9 y				≤1.5	
8-10.9 y	≤35				
1-11.9 y					≤3.4
11-11.9 y	≤40			≤1.5	
10-13.9 y		0.36-3.16	0.1-7.4		
12-13.9 y	≤40			≤1.5	≤3.4
14-17.9 y	≤40	0.41-2.34	0.5-3.9	≤3.6	≤7.8
Tanner Stage					
Stage I	≤8				
Stage II	≤24				
Stage III	≤28				
Stage IV	≤31				
Stage V	≤33				
Males					
Cord blood ^{10,11}	17-61				
1-10 d ^{10,11}	≤187				
1-3 mo ^{10,11}	72-344				
3-5 mo ^{10,11}	≤201				
5-7 mo ^{10,11}	≤59				
7-12 mo ^{10,11}	≤16				
1-5.9 y	≤5				
6-7.9 y	≤25				
5-9.9 y		0.44-1.78	≤5.3		
1-10.9 y				≤1.3	
8-10.9 y	≤42				
1-11.9 y					≤5.4
11-11.9 y	≤260			≤1.3	
10-13.9 y		0.53-3.33	0.7-52		
12-13.9 y	≤420			≤64	≤140
14-17.9 y	≤1000	1.05-2.91	18-111	4.0-100	8.0-210
Tanner Stage					
Stage I	≤5				
Stage II	≤167				
Stage III	21-719				
Stage IV	25-912				
Stage V	110-975				

^a Test code: 15983X, 36170X, 14966X.^b Test code: 36170X.^c Test code: 14966X.

Table 3. Testosterone Binding Proteins Reference Ranges^a

Age	SHBG, nmol/L		Albumin, g/dL
	Females	Males	
<3 y	Not established	Not established	
3-9 y	18-136	18-136	
10-13 y	17-123	17-123	
14-17 y	11-71	11-71	
Tanner Stage			
Stage I	38-114	39-155	
Stage II	24-90	33-135	
Stage III	22-112	21-72	
Stage IV	22-69	11-92	
Stage V	18-76	18-54	
Adult			
18-29 y	6-112	7-49	
30-39 y	14-102	8-48	
40-49 y	11-100	9-45	
50-59 y	17-78	18-47	
60-69 y	17-95	17-54	
70-79 y	21-90	23-65	
80-91 y	26-77	20-63	
>91 y	Not established	Not established	3.6-5.1

SHBG, sex hormone binding globulin.

^aTest code: 14966X.Table 4. Testosterone Levels and Intersex Disorders¹³

Condition	Genotype	External Genitalia	Testosterone
Complete androgen insensitivity syndrome	XY	female	normal
Partial androgen insensitivity syndrome	XY	ambiguous	normal
Complete gonadal dysgenesis	XY	female	absent
Partial gonadal dysgenesis	XY	ambiguous	decreased
5Alpha-reductase deficiency	XY	ambiguous	normal ^a
Complete testosterone biosynthetic defect	XY	female	absent
Partial testosterone biosynthetic defect	XY	ambiguous	decreased
Micro penis	XY	micro penis	decreased
Congenital adrenal hyperplasia	XX	ambiguous	increased
Klinefelter syndrome	XXY	small penis	decreased
Turner syndrome	XO	female	absent
45XO,46XY mosaicism		ambiguous	variable

^a Dihydrotestosterone absent.

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*The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payor being billed.

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14225 Newbrook Drive
PO Box 10841
Chantilly, VA 20153-0841
703-802-6900
800-336-3718

33608 Ortega Highway
San Juan Capistrano, CA 92690-6130
949-728-4000
800-642-4657
www.nicholsinstitute.com

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