

Test Summary

Testosterone, LC/MS/MS

Total (Women and Children):testcode1
Free and Total:testcode2
Free, Bioavailable, and Total:testcode3

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

Recent 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 recently 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 (Women and Children): 1 mL; 0.18 mL minimum

Free and Total Testosterone: 1 mL; 0.5 mL minimum

Free, Bioavailable, and Total Testosterone: 2.5 mL; 1.1 mL minimum

Method

- Total Testosterone (Women and Children)
 - 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 affinity constants for the binding of testosterone to SHBG and albumin
 - Bioavailable: calculated based on affinity constants for the binding of testosterone to SHBG and albumin
 - SHBG: extraction, chromatography, radioimmunoassay
 - 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.^{2,12} 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) or secondary hypogonadism (associated with decreased LH and FSH), or treatment of prostate cancer with gonadotropin releasing hormone analogs or antiandrogens.¹⁵ 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)* (ng/dL)	Free and Total†		Free, Bioavailable, and Total‡	
		% Free (percent)	Free (pg/mL)	Free (pg/mL)	Bioavailable (ng/dL)
Females					
18-29	≤44	0.7-2.3	≤6.3	0.3-5.4	0.8-12.3
30-39	4-39	0.5-2.3	≤6.3	0.3-3.5	0.7-8.2
40-49	4-34	0.5-2.3	≤6.2	0.2-3.8	0.6-7.8
50-59	3-46	0.6-2.6	≤6.9	0.1-1.9	0.1-5.0
60-69	3-42	0.5-2.0	≤1.9	0.1-1.9	0.1-3.2
70-84	3-20				
70-87		0.3-1.4	≤1.3		
70-89				0.2-1.7	0.4-3.2
Males					
18-29	189-1111	1.1-2.8	40-150	37-240	98-615
30-39	236-1076	1.0-3.1	31-160	43-203	110-510
40-49	217-1107	1.1-2.8	20-120	47-182	124-419
50-59	119-1104	0.9-2.5	11-141	43-200	108-506
60-69	168-796	1.5-2.2	55-90	no data	no data
70-79	117-819				
80-89	≤873				
70-87		1.0-2.5	39-150		
70-89				8.53	17-106

* testcode1, 2 and 3

† testcode2

‡ testcode3

Table 2. Testosterone Reference Ranges in Children and Adolescents

Age (years)	Total (LC/MS/MS)* (ng/dL)	Free and Total†		Free, Bioavailable, and Total‡	
		% Free (percent)	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				
1-7.9 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				
10-13.9 y		0.36-3.16	0.1-7.4		
11-11.9 y	≤40			≤1.5	≤3.4
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				
1-7.9 y	≤40				
5-9.9 y		0.44-1.78	≤5.3		
1-10.9 y				≤1.3	
8-10.9 y	≤42				
10-13.9 y		0.53-3.33	0.7-52		
11-11.9 y	≤260			≤1.3	≤5.4
12-13.9 y	≤420			≤64	≤140
14-17.9 y	≤1000	1.05-2.91	18-111	4-100	8-210
Tanner Stage					
Stage I	≤5				
Stage II	≤167				
Stage III	21-719				
Stage IV	25-912				
Stage V	110-975				

* testcode1, 2 and 3

† testcode2

‡ testcode3

Table 3. Testosterone Binding Proteins Reference Ranges*

Age	SHBG (nmol/L)	Albumin (g/dL)
Females		
Preterm infants	14-40	
Term infants	16-44	
2-8 y	41-137	
9-14 y	15-123	
Adult	17-120	
0-40 y		3.7-5.1
41-60 y		3.5-4.9
>60 y		3.2-4.6
Males		
Preterm infants	24-56	
Term infants	24-54	
2-8 y	29-141	
9-14 y	32-92	
Adult	7-50	
0-40 y		3.7-5.1
41-60 y		3.5-4.9
>60 y		3.2-4.6

SHBG, sex hormone binding globulin

* testcode3

Table 4. Testosterone Levels and Intersex Disorders¹⁴

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*
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

*Dihydrotestosterone absent.

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This test was developed and its performance characteristics determined by Quest Diagnostics Nichols Institute. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Performance characteristics refer to the analytical performance of the test.

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