Endocrinology

Test Selection
and
Interpretation

Fourth Edition
Delbert A. Fisher, MD
Editor

Quest Diagnostics®

Nichols Institute

The Quest Diagnostics Manual

Endocrinology Test Selection and Interpretation

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Forward

Quest Diagnostics Nichols Institute has been committed to providing state of the art endocrine testing for nearly 4 decades. During this time, we have introduced assays for most of the endocrine systems hormones, important metabolites and carrier proteins, free hormones, many hormone receptors, endocrine system autoantibodies, relevant chromosomal abnormalities, and selected genetic mutations. Our commitment to state of the art diagnostics and our dedication to quality and service have always included both adult and pediatric patients, and we have an ongoing program providing adult and pediatric reference range and clinical correlations data. Additionally, we have special procedures for identification and handling of pediatric samples, including reduced sample volume, whenever possible.

In this manual, we have consolidated detailed technical information for more than 150 of the endocrine and endocrine-related tests performed at Nichols Institute. The manual includes an overview of the physiology of each endocrine system, the diagnostic applications of each test, interpretation of test results, and a catalog of the endocrine-related dynamic or perturbation tests that facilitate diagnosis and patient management. For your convenience, we have included a guide for interconversion of conventional and SI units for our endocrine tests. In addition, we have indexed our endocrine tests in several formats to provide easier access to the information.

National Quest Diagnostics test codes are listed for each of the tests described in the Alphabetical Test section. Methodologies may differ for the non-Nichols Institute regional laboratory tests; information and reference ranges for these tests are available from your sales representative or regional laboratory personnel.

We trust that the manual will assist you in the diagnosis and management of your adult and pediatric patients and reiterate our commitment at Quest Diagnostics to provide you with the highest quality test results and ready availability of consultative services. Our laboratory staff and our Medical and Scientific Directors are available for consultation to help assure the most efficacious resolution of complex or confusing endocrine problems. We welcome your feedback and suggestions for improving our service to you.

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Delbert A. Fisher, MD

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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.

†This test is performed using a kit that has not been approved or cleared by the FDA. The analytical performance characteristics of this test have been determined by Quest Diagnostics Nichols Institute. This test should not be used for diagnosis without confirmation by other medically established means.

‡Refer to the Quest Diagnostics Nichols Institute Directory of Services for ordering information.

Reflex tests are performed at an additional charge and are associated with an additional CPT code.

Alphabetical Test Section

Test components are disclosed in the individual test write-ups in this section.

ACTH, Plasma 211X

Clinical Use

- Diagnose disorders of the hypothalamic-pituitary-adrenal system
- Differentiate Cushing's syndrome from normal when ACTH levels are low

Reference Range

	pg/mL
Men	7-50
Women	5-27
Children*	
Prepubertal	7-28
Pubertal	2-49

*Pediatric data from *Euro J Endocrin.* 1997; 137:635-647.

Interpretive Information

↑
- ACTH-secreting tumor
- Pituitary Cushing's disease
- Addison's disease
- Stress

↓
- Adrenal adenoma
- Adrenal carcinoma
- Secondary adrenal insuffiency

Clinical Background

Adrenocorticotropic hormone (ACTH) is a 39-amino acid polypeptide secreted mainly by the anterior pituitary gland. The hypothalamus controls pituitary ACTH secretion by means of corticotropin releasing hormone (CRH), a 41-amino acid peptide released in response to pain, anxiety, and stress. There also is a diurnal variation. Cortisol exerts negative feedback control on the secretion of ACTH at the pituitary gland and hypothalamic levels.

Plasma ACTH measurements are useful in diagnosing disorders of the hypothalamic-pituitary-adrenal system.

Method

- Immunochemiluminometric assay (ICMA)
- Detects intact ACTH
- Analytical sensitivity: 5 pg/mL

Specimen Requirements

1.5 mL frozen EDTA plasma

0.3 mL minimum

Collect samples between 7 and 10 AM.

Adrenal Antibody 4645X

Clinical Use

- Confirm autoimmune nature of Addison's disease
- Identify adrenal gland involvement in polyglandular autoimmune syndrome

Reference Range

Negative
Titer: <10

Interpretive Information

- Polyglandular autoimmune disease, type I
- Polyglandular autoimmune disease, type II
- Isolated autoimmune Addison's disease

Clinical Background

Autoimmune Addison's disease is characterized by the presence of serum autoantibodies to adrenal antigens. Recent studies have identified the adrenal biosynthetic enzymes $P450_{c21}$ and $P450_{c17}$ as 2 of the synthetic antigens. Immunofluorescence is useful for adrenal autoantibody detection. Addison's disease is seen as part of 2 types of polyglandular autoimmune syndromes (PAS): type I includes candidiasis and hypoparathyroidism and type II includes autoimmune thyroiditis and type 1 diabetes. Type II is more frequent. Measurement of other glandular autoantibodies may provide useful diagnostic and management information.

Method

- Immunofluorescence assay (IFA)
- Titer provided if antibody is present (at additional charge)

Specimen Requirements

2 mL refrigerated serum
0.5 mL minimum

No additive red top preferred
SST red top acceptable

Aldosterone, 24-Hour Urine 19552X

Clinical Use

- Assess adrenal aldosterone production
- Diagnose primary hyperaldosteronism due to adrenal adenomas or bilateral adrenal hyperplasia

Reference Range

Aldosterone	µg/24-h
Random sodium diet	
2-7 y	≤5.7
8-11 y	≤10.2
12-16 y	≤15.6
Adults	2.3-21.0
Post-Florinef® suppression or IV saline infusion	≤5

Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Primary hyperaldosteronism
- Secondary hyperaldosteronism
- Very low sodium diet
- Pregnancy
- Bartter syndrome

↓
- Congenital adrenal hyperplasia
- Congenital deficiency of aldosterone synthetase
- Adrenal hypoplasia
- Hyporeninemic hypoaldosteronism
- Addison's disease
- Very high sodium diet

Results affected by recent sodium intake

Clinical Background

Aldosterone, an adrenal cortex hormone, is the major regulator of sodium and potassium excretion. Production is modulated largely by the renin-angiotensin system in response to changes in sodium balance and renal blood flow. Urine aldosterone excretion is commonly increased in secondary (high renin) states of heart failure or liver or renal disease. In primary hyperaldosteronism due to autonomous adrenal secretion, urine aldosterone is increased and plasma renin is suppressed. Aldosterone excretion is reduced in Addison's disease and in hyporeninemic hypoaldosteronism caused by renal disorders. The major urinary aldosterone metabolite is aldosterone-18 glucuronide. Measurement of aldosterone in urine, corrected for creatinine, provides an estimate of secretion rate over a 24-hour period.

Method

- Acid hydrolysis, extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 1 µg/L
- Creatinine concentration also reported.

Specimen Requirements

5 mL refrigerated aliquot of a 24-h urine; 0.8 mL minimum

Collect urine in 10 g of boric acid. Refrigerate during collection. Record 24-h volume on vial and request form.

Aldosterone, LC/MS/MS, Serum 17181X

Clinical Use

- Assess adrenal aldosterone production
- Diagnose primary hyperaldosteronism
- Differential diagnosis of fluid and electrolyte disorders

Reference Range

	ng/dL
Adults	
Upright (8-10 AM)	≤28
Upright (4-6 PM)	≤21
Supine (8-10 AM)	3-16
Children	
Premature infants (31-35 wks)*	≤144
Term infants*	≤217
1-12 mo	2-70
1-4 y	2-37
5-9 y	≤9
10-13 y	≤21
14-17 y	≤35
Tanner II-III	
Males	1-13
Females	2-20
Tanner IV-V	
Males	3-14
Females	4-32

*Data from *J Clin Endocrinol Metab.* 1992;75: 1491-1496 and *J Clin Endocrinol Metab.* 1989; 69:1133-1136.

Interpretive Information

↑
- Primary hyperaldosteronism
- Secondary hyperaldosteronism
- Very low sodium diet
- Pregnancy
- Bartter syndrome

↓
- Congenital adrenal hyperplasia
- Aldosterone synthetase deficiency
- Very high sodium diet
- Addison's disease
- Hyporeninemic hypoaldosteronism

Levels are affected by recent sodium intake and posture.

Clinical Background

Aldosterone is the major sodium-retaining hormone produced by the adrenal gland. Secretion is normally regulated by renin-angiotensin system monitoring of extracellular fluid volume. A low- salt diet will decrease volume, increase renin-angiotensin, and increase aldosterone. Potassium is a secondary regulator. Diseases of the heart, liver, and kidneys commonly increase renin, leading to secondary aldosteronism. Primary hyperaldosteronism due to autonomous secretion from an adrenal adenoma or secondary to bilateral hyperplasia produces sodium retention, hypertension, and renal potassium wasting. An elevated serum aldosterone with low renin (PRA or direct renin) in the face of hypokalemia is diagnostic of primary hyperaldosteronism. Aldosterone deficiency occurs in Addison's disease (associated with a high renin) and in patients with isolated hypoaldosteronism, which may be hyporeninemic (eg, diabetes) or hyperreninemic (eg, AIDS). In children, adrenal biosynthetic enzyme deficiencies may produce aldosterone deficiency.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1.0 ng/dL

Specimen Requirements

1.0 mL refrigerated serum (no additive red top tube); 0.25 mL minimum

SST red top tube unacceptable.

Draw upright samples ½ hour after patient sits up.

Aldosterone/PRA Ratio 11183Z

Clinical Use

- Diagnose primary hyperaldosteronism

Reference Range

Aldosterone/PRA Ratio

1.5-18.2 ng/dL / ng/mL/h

See ***Aldosterone, LC/MS/MS, Serum*** for aldosterone reference ranges.

See ***Plasma Renin Activity (PRA)*** for PRA reference ranges.

Interpretive Information

↑ Ratio

- Primary hyperaldosteronism

Normal or decreased ratio

- Secondary hyperaldosteronism
- Hyporeninemic hypoaldosteronism
- Pregnancy
- Bartter syndrome
- Congenital adrenal hyperplasia
- Aldosterone synthetase deficiency
- Addison's disease

Levels are affected by recent sodium intake and posture.

Clinical Background

Primary hyperaldosteronism due to autonomous secretion from an adrenal adenoma or secondary to bilateral hyperplasia produces sodium retention, hypertension, and renal potassium wasting. An elevated serum aldosterone with low renin (PRA or direct renin) in the face of hypokalemia is diagnostic of primary hyperaldosteronism. Diseases of the heart, liver, and kidneys commonly increase renin, leading to secondary aldosteronism. A normal aldosterone/PRA ratio in the presence of hyperkalemia is consistent with hyporeninemic hypoaldosteronism.

Method

Aldosterone

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1.0 ng/dL

PRA

- Angiotensin I generation, radioimmunoassay (RIA)
- Analytical sensitivity: 0.37 ng/mL/h

Specimen Requirements

3.0 mL frozen EDTA plasma

1.0 mL minimum

Do not refrigerate.

Patient preparation: ambulatory for 30 minutes prior; moderate sodium diet; avoid medications for 3 weeks prior.

Alkaline Phosphatase, Bone Specific

29498X

Clinical Use

- Therapeutic monitoring of postmenopausal osteoporosis and Paget's disease

Reference Range

	Males	Females
Adults	µg/L	µg/L
18-29 y	8.4-29.3	4.7-17.8
30-39 y	7.7-21.3	5.3-19.5
40-49 y	7.0-18.3	5.0-18.8
50-68 y	7.6-14.9	
50-76 y		5.6-29
Children		
2-24 mo	25.4-124.0	25.4-124.0
6-9 y	41.0-134.6	41.0-134.6
10-13 y	43.8-177.4	24.2-154.2
14-17 y	13.7-128.0	10.5-75.2

Pediatric data from *Int J Biol Markers.* 1996;11:159-164.

Interpretive Information

- Hyperthyroidism
- Osteomalacia
- Osteoporosis
- Other metabolic bone diseases
- Paget's disease (osteitis deformans)
- Primary hyperparathyroidism

- Response to therapy

Clinical Background

Bone-specific alkaline phosphatase (BSAP) is one of a group of alkaline phosphatase enzymes produced in various tissues including bone, liver, placenta, intestine, spleen, and kidney. The bone-specific isoenzyme is synthesized and released by osteoblastic cells during the process of bone formation. The processes of bone formation and bone dissolution are linked. Thus, BSAP levels reflect osteoblast cellular activity due to either increased bone formation or osteoblastic stimulation associated with excessive bone destruction.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.1 µg/L

Specimen Requirements

1 mL frozen serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Alpha Subunit 8658X

Clinical Use

- Diagnose and manage pituitary, placental, and pancreatic tumors

Reference Range

	ng/mL
Men	≤0.6
Women	
Premenopausal	≤1.5
Postmenopausal	0.9-3.3
Hypothyroid	≤3.7
Pregnant or hCG-producing tumors	1.8-360*

*Varies with concentration of hCG

Interpretive Information

- Pregnancy
- Choriocarcinoma
- Hydatidiform mole
- Pituitary adenomas (selected)

- Hypopituitarism

Clinical Background

The pituitary glycoprotein hormones–thyroid stimulating hormone (TSH), luteinizing hormone (LH), and follicle stimulating hormone (FSH)–are comprised of identical alpha subunits and unique beta subunits that confer biological specificity. Some pituitary adenomas unassociated with a clinical syndrome of hormonal over secretion produce the alpha subunit, which serves as a useful tumor marker. Measurement of the alpha subunit can be useful in differentiating TSH-secreting pituitary adenomas, in which the ratio of alpha-to-intact TSH serum concentrations is >1, from thyroid hormone resistance syndromes, in which the ratio is ≤1.

Gonadotropin-secreting pituitary adenomas producing excessive alpha subunit have been reported. Non-pituitary tumors, eg, choriocarcinoma and carcinoid tumors, secreting the alpha subunit have also been described.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 0.1 ng/mL at 90% B/Bo
- Analytical specificity: cross-reactivity is 1.4% with intact hCG, <0.01% with beta subunit hCG, 4.5% with LH, 3.4% with FSH, and 2.0% with TSH

Specimen Requirements

2 mL refrigerated serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

3α-Androstanediol Glucuronide (3α-diol G) 5276X

Clinical Use

- Marker for disorders of peripheral androgen formation and action, such as in hirsutism and acne

Reference Range

	ng/dL
Men	260-1500
Women	60-300
Children	
Prepubertal	10-60
Tanner II-III	
Males	19-164
Females	33-244

Tanner stage data from *J Clin Endocrinol Metab.* 1991;72:46-50.

Interpretive Information

- ↑ Idiopathic hirsutism (females)
- ↑ PCO-hirsutism (females)
- ↑ Acne (females)
- ↓ 5α-reductase deficiency (males)

Clinical Background

3α-Androstanediol glucuronide (3α-diol G) is a metabolite of dihydrotestosterone (DHT) produced in androgen responsive tissues, such as hair follicles. 3α-diol G is a marker of a peripheral androgen formation and action.

The most important application of this assay is in hirsute states. Patients with hirsutism of either the idiopathic type or secondary to polycystic ovarian diseases (PCO) may have markedly elevated levels. 3α-diol G has also been shown to be a marker of therapeutic response in congenital adrenal hyperplasia (CAH).

Method

- Extraction, enzyme digestion, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 5 ng/dL

Specimen Requirements

2 mL refrigerated serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Androstenedione, LC/MS/MS 17182X

Clinical Use

- Diagnose hirsutism, polycystic ovarian disease, virilization, and congenital adrenal hyperplasia

Reference Range

	ng/dL
Men	
18-30 y	50-220
31-50 y	40-190
51-60 y	50-220
Women	
Follicular	35-250
Midcycle	60-285
Luteal	30-235
Postmenopausal	20-75
Children	
1-12 m	6-78
1-4 y	5-51
5-9 y	6-115
10-13 y	12-221
14-17 y	22-225
Tanner II-III	
Males	17-82
Females	43-180
Tanner IV-V	
Males	57-150
Females	7-68

Pediatric data from *J Clin Endocrinol Metab.* 1991;73:674-686 and *J Clin Endocrinol Metab.* 1989;69:1133-1136.

Interpretive Information

- Adrenal tumors
- Cushing's disease
- Congenital adrenal hyperplasia
- Polycystic ovarian disease
- Idiopathic hirsutism

- Addison's disease

Clinical Background

Androstenedione is an androgenic steroid hormone produced by both the adrenal cortex and the gonads. In males, the compound's activity is relatively unimportant, compared with the predominant testicular androgen testosterone. In females, androstenedione–and the testosterone and dihydrotestosterone to which it is metabolized–normally contribute to the growth of sexually dependent axillary and pubic hair. In women, the concentration of androstenedione varies with the menstrual cycle, with the ovaries and adrenals contributing equally during the follicular phase. At mid-cycle, increased ovarian androstenedione secretion accounts for 70% of its overall production.

Excessive androstenedione production may cause hirsutism and contribute to virilization. This can occur in the polycystic ovary syndrome, idiopathic hirsutism, ovarian and adrenal neoplasms, and congenital adrenal hyperplasia due to 21-hydroxylase or 17β-hydroxylase deficiencies. In hirsute patients with an elevated androstenedione concentration, serial measurements are useful to monitor therapy, since clinical improvement typically lags behind the biochemical response by months.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 5 ng/dL

Specimen Requirements

1 mL refrigerated serum

0.25 mL minimum

No additive red top tube

SST red top unacceptable

Angiotensin II 36718X

Clinical Use

- Differential diagnosis of renal hypertension

Reference Range

Adults	10-50 ng/L

Interpretive Information

- High renin hypertension
- Renin-secreting juxta-glomerular renal tumor
- Volume depletion
- Congestive heart failure
- Cirrhosis
- Angiotensin II receptor blocking drug therapy

- Anephric patients
- Primary aldosteronism
- Cushing's syndrome
- ACE inhibitor therapy

Clinical Background

Angiotensin II is the primary regulator of renal aldosterone secretion and a potent vasoconstrictor. It is generated through the renin angiotensin system (RAS): circulating angiotensinogen is cleaved by renin to form angiotensin I, which is then converted to angiotensin II via angiotensin converting enzyme (ACE). The rate-limiting step in the RAS is renin secretion by the renal juxtaglomerular cells, modulated by renal blood flow. Low renal blood flow and low perfusion pressure increases renin secretion, stimulating angiotensin II and aldosterone production with a resulting increase in blood pressure and renal sodium retention. These changes then produce inhibition of renin secretion and complete the feedback control loop.

Method

- Radioimmunoassay (RIA)

Specimen Requirements

1 mL frozen EDTA plasma

0.3 mL minimum

Arginine Vasopressin, Plasma 252X

Clinical Use

- Diagnose central diabetes insipidus (DI)
- Differential diagnosis of central DI vs nephrogenic DI
- Diagnose syndrome of inappropriate ADH secretion (SIADH)

Reference Range

1.0-13.3 pg/mL
Note: 2.5 pg = 1 µU

Interpretive Information

↑
- SIADH
- Ectopic ADH syndrome
- Nephrogenic DI
- Phenothiazine, carbamazepine

↓
- Central DI

Clinical Background

Arginine vasopressin (AVP), or antidiuretic hormone (ADH), is a nonapeptide produced by the hypothalamus and released from the posterior pituitary in response to extracellular fluid hyperosmolarity and hypovolemia. AVP promotes concentration of the urine by increasing water reabsorption in the kidney tubules. Inadequate AVP action causes diabetes insipidus (DI), a syndrome characterized by nonglycosuric polyuria, polydipsia, and dehydration. Central DI refers to insufficient AVP release due to diseases of the hypothalamus, pituitary stalk, and pituitary gland. Nephrogenic DI is the result of impaired renal responsiveness to AVP and may be congenital or due to renal disease, hypokalemia, hypercalcemia, systemic disorders (eg, multiple myeloma and amyloidosis), or drugs (eg, lithium or demeclocycline and ethanol).

DI diagnosis is based on the presence of hyperosmolar serum with inappropriately dilute urine. Central and nephrogenic DI can be differentiated by measuring the plasma AVP level and interpreting it in light of the simultaneous plasma osmolality.

The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is manifest by hyponatremia and inappropriately concentrated urine. The diagnosis is confirmed by plasma or urine AVP levels inappropriate for serum osmolality.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 1.0 pg/mL

Specimen Requirements

4 mL frozen EDTA plasma

1.1 mL minimum

Draw blood in prechilled tube; centrifuge immediately in refrigerated centrifuge.

Arginine Vasopressin (AVP) and Osmolality, Random Urine

36572X

Clinical Use

- Diagnose central diabetes insipidus (DI)
- Differential diagnosis of central DI vs nephrogenic DI
- Confirm syndrome of inappropriate ADH secretion (SIADH) diagnosis

Reference Range

AVP	1-112 pg/mL
Osmolality	50-1200 mOsm/kg

Interpretive Information

- Low AVP and urine osmolality = central DI
- High AVP and low urine osmolality = nephrogenic DI
- Normal AVP despite low serum osmolality = SIADH; in SIADH, the kidney responds normally to AVP

Clinical Background

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is a 9-amino acid polypeptide synthesized by the hypothalamus and stored and secreted by the posterior pituitary. Secretion is regulated by osmoreceptors and volume receptors and is increased by dehydration or increased blood osmolarity. Conversely, secretion is decreased by increased blood volume or decreased blood osmolarity. AVP acts primarily on the kidney, where it exerts an antidiuretic effect.

AVP plasma concentrations correlate with osmolarity. Significant amounts of AVP are filtered via the glomerulus and appear in urine. Concentrations are higher in urine than in plasma. Urine AVP provides an integrated view of recent blood levels. Urine AVP determinations are useful in the differential diagnosis of hyponatremic states.

Method

AVP

- Radioimmunoassay (RIA)
- Analytical sensitivity: 1.0 pg/mL

Osmolality

- Freezing point depression

Specimen Requirements

3 mL refrigerated random urine

1.2 mL minimum

Do not use a preservative.

CAH (21-Hydroxylase Deficiency) Common Mutations 14755X

Clinical Use

- Confirm diagnosis of 21-hydroxylase deficiency (21-OHD)
- Screen for 21-OHD carrier status in at-risk individuals
- Prenatal diagnosis of 21-OHD

Reference Range

Negative (no mutations detected)

Interpretive Information

Positive (mutations detected)

- 21-hydroxylase deficiency (carrier or affected)

Clinical Background

21-Hydroxylase deficiency, the most common cause of congenital adrenal hyperplasia (CAH), is an autosomal recessive disorder caused by mutations or rearrangements in the *CYP21A2* gene on chromosome 6. The deficiency is characterized by decreased cortisol and increased androgen blood levels. Severe reduction in 21-hydroxylase activity causes classic CAH, the simple virilizing form (25% of cases), and/or the salt-wasting form, which is further characterized by decreased aldosterone levels. Onset occurs prenatally and, if detected prenatally, it can be treated to reduce virilization in affected females. Non-classic CAH, on the other hand, presents postnatally with signs of hyperandrogenism.

Testing for the more common mutations can detect both relevant mutations in 81% and 1 mutation in about 18% of affected individuals. Preparation for prenatal testing requires mutation analysis of parents and/or an affected offspring.

Method

- Polymerase chain reaction (PCR) and DNA mini-sequencing
- Common mutations sought: P30L; Intron 2 "g"; G110del8nt; I172N; exon 6 cluster of I236N, V237E & M239K; V281L; F306+1nt; Q318X; R356W; and P453S
- Deduction of deletions and recombinations between *CYP21A2* and its pseudogene

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube.

For prenatal testing, submit amniotic fluid, CVS sample, or cultured cells from either source.

CAH (21-Hydroxylase Deficiency) Rare Mutations 16072X

Clinical Use

- Confirm diagnosis of 21-hydroxylase deficiency (21-OHD)
- Determine 21-OHD carrier status in at-risk individuals
- Prenatal diagnosis of 21-OHD

Reference Range

Negative (no mutations detected)

Interpretive Information

Positive (mutations detected)

- 21-hydroxylase deficiency (carrier or affected)

Clinical Background

21-Hydroxylase deficiency, the most common cause of congenital adrenal hyperplasia (CAH), is an autosomal recessive disorder caused by mutations or rearrangements in the *CYP21A2* gene on chromosome 6. The deficiency is characterized by decreased cortisol and increased androgen blood levels. Severe reduction in 21-hydroxylase activity causes classic CAH, the simple virilizing form (25% of cases), and/or the salt-wasting form, which is further characterized by decreased aldosterone levels. Onset occurs prenatally and, if detected prenatally, it can be treated to reduce virilization in affected females. Non-classic CAH, on the other hand, presents postnatally with signs of hyperandrogenism.

This test is appropriate for affected individuals in whom only 1 or no mutations were detected in the CAH (21-Hydroxylase Deficiency) Common Mutations test. It is also appropriate for carrier testing in family members of such individuals, once a rare mutation has been identified. About 1% of affected individuals have rare mutations on both chromosomes, and about 18% have a rare mutation on only 1 chromosome.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Entire *CYP21A2* gene sequenced

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube.

For prenatal testing, submit amniotic fluid, CVS sample, or cultured cells from either source.

Call 1-866-GENEINFO (1-866-436-3436) before collecting or submitting samples.

CAH Panel 1 (21-Hydroxylase vs 11β-Hydroxylase Deficiency) 15269X

Clinical Use

- Diagnose 21-hydroxylase or 11β-hydroxylase deficiency

Reference Range

Androstenedione	*
Cortisol	*
11-Deoxycortisol	*
17-Hydroxyprogesterone	*
Testosterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

17-OH progesterone/ 11-deoxycortisol ratio*	21-OH: >6
11-Deoxycortisol/ cortisol ratio*	11β-OH: >100 (except prematures)
Androstenedione	↑
Testosterone	↑

*See Test Application and Interpretation, Disorders of Adrenal Function, Tables 6-8.

Clinical Background

21-Hydroxylase deficiency (21-OHD, $P450_{c21}$) accounts for 90% to 95% of CAH cases. Classic 21-OHD is characterized by markedly diminished or absent 21-hydroxylase activity. Affected individuals typically present at birth or in the neonatal period with either a virilizing or salt-wasting form. Female infants with virilizing 21-OHD have varying degrees of masculinization ranging from clitoral enlargement to complete development of male external genitalia. Male infants have normal male genitalia. Symptoms of hyponatremia, hyperkalemia, volume depletion, and decreased blood pressure generally appear within the first 2 weeks of life in patients with the salt-wasting form.

Nonclassic 21-OHD is characterized by marginally decreased 21-hydroxylase activity. Affected females usually present in childhood or post puberty with evidence of androgen excess (eg, increased pubic hair growth and/or clitoral enlargement). In women, nonclassic 21-OHD is frequently confused with polycystic ovary syndrome. Diagnosis may require ACTH stimulation.

11β-Hydroxlase deficiency (*CYP11B1*, 11βHSD) accounts for 5% to 8% of CAH cases. Salt-wasting does not occur; however, virilization of female fetuses can be severe. Elevated blood pressure manifests early in life in approximately two-thirds of patients and, along with elevated deoxycorticosterone and 11-deoxycortisol levels, clinically distinguishes 11β- from 21-hydroxylase deficiency. A late-onset form that typically presents with signs of androgen excess is analogous to nonclassic 21-OHD.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

0.6 mL refrigerated serum

0.3 mL minimum

No additive red top tube

An early morning specimen is preferred. Specify age and sex on test request form.

CAH Panel 3 (Aldosterone Synthase Deficiency) 15273X

Clinical Use

- Diagnose aldosterone synthase deficiency

Reference Range

Androstenedione	*
11-Deoxycortisol	*
18-Hydroxycorticosterone	*
17-Hydroxyprogesterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

18-OH corticosterone/ aldosterone ratio*	>40

*See Test Application and Interpretation, Disorders of Adrenal Function, Tables 6-8.

Clinical Background

Aldosterone synthase deficiency (*CYP11B2*, $P450_{c11}ase$) is a rare form of CAH in which only aldosterone synthesis is affected. Two forms have been identified; type I is characterized by decreased, and type II by increased, 18-hydroxycorticosterone. Infants usually present in the neonatal period with failure to thrive, recurrent dehydration secondary to salt-wasting, decreased blood pressure, and acidosis.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

1.8 mL refrigerated serum

0.8 mL minimum

No additive red top tube

An early morning specimen is preferred.

Specify age and sex on test request form.

CAH Panels 4 and 8 (17-Hydroxylase Deficiency) 15274X 15279X

Clinical Use

- Diagnose 17-hydroxylase deficiency in females (15274X)
- Diagnose 17-hydroxylase deficiency in males (15279X)

Reference Range

CAH Panel 4 (15274X)

Aldosterone	*
Corticosterone	*
Cortisol	*
17-Hydroxyprogesterone	*
Progesterone	*
Estradiol	*

CAH Panel 8 (15279X)

Aldosterone	*
Corticosterone	*
Cortisol	*
17-Hydroxyprogesterone	*
Progesterone	*
Testosterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

CAH Panel 4 (15274X)

Progesterone/17-OH progesterone ratio*	>6
Aldosterone	↓
Corticosterone	↑
Cortisol	↓
Estradiol	↓

CAH Panel 8 (15279X)

Progesterone/17-OH progesterone ratio*	>6
Aldosterone	↓
Corticosterone	↑
Cortisol	↓
Testosterone	↓

*See Test Application and Interpretation, Disorders of Adrenal Function, Tables 6-8

Clinical Background

17α-Hydroxylase deficiency (*CYP17*, $P450_{c17}$) accounts for approximately 1% of all CAH cases, with an estimated incidence of 1:50,000 newborns. The *CYP17* gene encodes an enzyme that catalyzes both 17α-hydroxylation and 17,20-lyase reactions. Isolated deficiency of either activity has been reported; however, a combined deficiency in which there is failure of catalysis of both reactions is the most common form. Affected individuals have decreased levels of cortisol, androgens, and estrogens and typically present during adolescence with primary amenorrhea and/or lack of secondary sexual characteristics. Males (XY) may be detected at birth with pseudohermaphroditism (ie, female external genitalia, absence of uterus and fallopian tubes, and intra-abdominal testes). At the time of diagnosis, individuals are usually found to be hypertensive and hypokalemic (low renin, low aldosterone). All such individuals should be tested for 17-hydroxylase deficiency.

Subjects with combined 21- and 17-hydroxylase/17,20-lyase deficiencies with or without Antley-Bixler syndrome (type 2) have been shown to have the P450 oxidoreductase gene mutation.

Method

CAH Panel 4 (15274X)

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

CAH Panel 8 (15279X)

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

CAH Panel 4 (15274X)

1.2 mL refrigerated serum

0.6 mL minimum

CAH Panel 8 (15279X)

0.8 mL refrigerated serum

0.4 mL minimum

No additive red top tube

An early morning specimen is preferred. Specify age, sex, and time of day sample was collected on test request form.

CAH Panel 6 (StAR Deficiency) 15276X

Clinical Use

- Diagnose StAR deficiency

Reference Range

Androstenedione	*
Cortisol	*
DHEA	*
Pregnenolone	*

*See individual assays for age-related reference ranges.

Interpretive Information

Androstenedione	↓
Cortisol	↓
DHEA	↓
Pregnenolone	↓

Clinical Background

Steroid acute regulatory protein (StAR) deficiency is responsible for congenital lipoid adrenal hyperplasia, a defect of cholesterol transport resulting in deficiency of all adrenal steroids. It is the rarest of the congenital adrenal steroid defects and has been fatal in two-thirds of the reported cases. The defect was thought to reside in *CYP11A*, the gene that codes for the cholesterol side-chain cleavage enzyme. However, recent molecular studies indicate the defect resides on chromosome 8 in the *StAR* gene, which encodes a phosphoprotein that enhances cholesterol transport from the outer to inner mitochondrial membrane. Affected individuals present in the neonatal period with severe adrenal insufficiency manifested by failure to thrive, vomiting, diarrhea, hyponatremia, and hypokalemia. Males typically have normal female external genitalia.

Method

- Extraction, radioimmunoassay (RIA)
- Extraction, chromatography, RIA
- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

1.6 mL refrigerated serum

1.1 mL minimum

No additive red top tube

An early morning specimen is preferred.

Specify age and sex on test request form.

CAH Panel 6B (Comprehensive Screen) 10299X

Clinical Use

- Diagnosis and differential diagnosis of congenital adrenal hyperplasia (CAH)

Reference Range

Androstenedione	*
Cortisol	*
DHEA	*
Deoxycorticosterone	*
11-Deoxycortisol	*
17-OH pregnenolone	*
17-OH progesterone	*
Progesterone	*
Testosterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

Results vary depending on the defect (21-OH, 11β-OH, 17α-OH, 17,20-lyase, 3β-HSD, StAR, or *CYPOR* deficiency). See Test Application and Interpretation, Disorders of Adrenal Function, Tables 6-8 for more information.

Clinical Background

Classic congenital adrenal hyperplasia (CAH) manifests in the neonatal period with impaired cortisol synthesis caused by genetic mutations that result in deficient activity of 1 of the adrenal biosynthetic enzymes. The most common form, accounting for 90% to 95% of CAH patients, is 21-hydroxylase (P-450 *CYP21A2*) deficiency; 11β-hydroxylase (P-450 *CYP11B1*) deficiency accounts for another 5% to 8%. 3β-Hydroxysteroid dehydrogenase (*HSD3B2*), aldosterone synthase (*CYP11B2*), steroid acute regulatory protein (*StAR*), and P-450 oxidoreductase (*CYPOR*) deficiencies constitute the remainder of cases in infancy. 17-hydroxylase deficiency usually presents at the time of puberty with hypertension and hypokalemia due to decreased 17α-hydroxylation of pregnenolone and progesterone in the adrenal and increased production of mineralocorticoids. Gonadal steroid production also is blocked, and LH and FSH levels are increased. Females have primary amenorrhea and absent sexual characteristics, and males present with complete pseudohermaphroditism (external female genitalia, no uterus, no fallopian tubes).

Method

- Extraction, radioimmunoassay (RIA)
- Extraction, chromatography, RIA
- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

2.4 mL refrigerated serum

1.5 mL minimum

No additive red top tube

An early morning specimen is preferred.

Specify age and sex on test request form.

CAH Panel 7 (21-Hydroxylase Deficiency Therapeutic Monitoring) 15277X

Clinical Use

- Monitor therapy in patients with 21-hydroxylase deficiency

Reference Range

Androstenedione	*
17-OH progesterone	*
Testosterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

Androstenedione	N or ↑
17-OH progesterone	N or ↑
Testosterone	N or ↑

Clinical Background

21-Hydroxylase deficiency is the most common cause of congenital adrenal hyperplasia (CAH). Selected steroids and steroid precursors, along with blood pressure, growth rate, skeletal age, and renin levels, are helpful when monitoring patients with this disorder.

Due to diurnal rhythm of 17-hydroxyprogesterone secretion, serial measurements should be standardized to the time of day and same conditions (eg, fasting 8 AM specimen prior to taking medications). Androstenedione measurements become less useful for monitoring purposes after adrenarche.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

0.4 mL refrigerated serum

0.2 mL minimum

No additive red top tube

An early morning specimen is preferred.

Specify age and sex on test request form.

CAH Panel 9 (3β-Hydroxysteroid Dehydrogenase Deficiency) 15280X

Clinical Use

- Diagnose 3β-hydroxysteroid dehydrogenase deficiency

Reference Range

Androstenedione	*
Cortisol	*
DHEA	*
17-OH pregnenolone	*
17-OH progesterone	*

*See individual assays for age-related reference ranges.

Interpretive Information

17-OH pregnenolone/ 17-OH progesterone	>40
DHEA/androstenedione	>30
Androstenedione	↓
Cortisol	↓

Clinical Background

3β-Hydroxysteroid dehydrogenase deficiency (*HSD3B2*) is a rare form of CAH characterized by increased levels of pregnenolone, 17-hydroxy-pregnenolone, and DHEA and decreased levels of all other adrenal steroids. Affected individuals usually present in infancy with signs of adrenal insufficiency. Female infants will typically have mild virilization. Phenotypic variation in male infants may range from hypospadias to complete male pseudohermaphroditism.

Method

- Extraction, radioimmunoassay (RIA)
- Extraction, chromatography, RIA
- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Specimen Requirements

1.0 mL refrigerated serum

0.5 mL minimum

No additive red top tube

An early morning specimen is preferred.

Specify age and sex on test request form.

CAH Panel 11, Neonatal Random Urine

10046X

Clinical Use

- Diagnose congenital adrenal hyperplasia (mild as well as severe forms) in the newborn

Reference Range

Steroid pediatric reference ranges: see Table 9*

Precursor-product ratio pediatric reference ranges: see Table 10*

Interpretive Information

See Table 10* for deficiency-specific pattern of precursor-product ratios for diagnosis of:

- 21-hydroxylase deficiency
- 17-hydroxylase deficiency
- 11β-hydroxylase deficiency
- 3β-hydroxysteroid dehydrogenase deficiency

*See Test Application and Interpretation, Disorders of Adrenal Function, section.

Clinical Background

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterized by deficiency of any 1 of 4 enzymes required for cortisol synthesis in the adrenal gland. Clinical symptoms vary according to the particular enzyme deficiency and include developmental abnormalities of the external genitalia and salt wasting (sodium and potassium imbalance). Differential diagnosis and treatment in the neonatal period is necessary to minimize morbidity and mortality.

CAH diagnosis is based on clinical findings, biochemical and hormonal test results, karyotyping, and sometimes mutation analysis. Although single hormone levels may be useful, ACTH stimulation tests are frequently required. In some cases, hormonal precursor-product ratios are needed to establish the definitive diagnosis. The precursor (ie, substrate of the deficient enzyme) is often elevated, and the product is decreased, leading to an elevated ratio. The differential diagnosis is made based on the pattern of precursor-product ratios.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Report includes 34 steroid measurements, 11 ratios, and interpretation
- Analytical sensitivity: see Table 9*

Specimen Requirements

7.0 mL frozen random urine

2.1 mL minimum

Use a premature infant diaper to collect a random urine sample: 1) remove the wet diaper from the baby; 2) remove and discard the top liner of the diaper; 3) insert the wetted diaper pad into a 20 cc syringe barrel; 4) replace the syringe plunger and press down to squeeze out urine; 5) mix well and freeze without preservatives.

Calcitonin 30742X

Clinical Use

- Diagnose and monitor medullary thyroid carcinoma

Reference Range

	pg/mL
Men	≤10
Women	≤5
Children	
<6 mo*	≤41
6 mo-3 y*	≤14
3-17 y	≤6

*From *Clin Chem.* 2004;50:1828-1829.

- ↑ Medullary carcinoma of the thyroid (1/3 have normal basal levels and require provocative test to reveal abnormal levels)
- Lung, breast, pancreatic cancer (some patients)
- Pancreatitis
- Thyroiditis
- Renal failure
- Zollinger-Ellison syndrome
- Pernicious anemia
- Pregnancy (term)
- Newborn infants

- ↓ Thyroid agenesis

Clinical Background

Calcitonin is a 32-amino acid polypeptide produced by parafollicular or C cells in the thyroid. Secretion of calcitonin is stimulated by calcium. Calcitonin decreases osteoclastic bone resorption, but the physiological role in man is uncertain.

Calcitonin measurement is indicated for the diagnosis and follow-up of patients with medullary thyroid carcinoma (MTC), the majority of whom produce the hormone. Settings in which the test is appropriate include 1) thyroid nodule in patients with symptoms potentially attributable to hypercalcitoninemia, 2) screening of individuals in families with known or suspected MTC or the multiple endocrine neoplasia syndrome types IIa or IIb, and 3) follow-up of patients with known MTC. Screening family members with normal basal calcitonin levels should include additional calcitonin measurements after pentagastrin and/or calcium stimulation (see Dynamic Tests).

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 2 pg/mL

Specimen Requirements

1.0 mL frozen serum

0.5 mL minimum

No additive red top

Overnight fasting is preferred.

Calcium, Ionized 306X

Clinical Use

- Diagnose hypo- and hypercalcemia

Reference Range

	mg/dL
Adults	4.8-5.6
Children	
8 mo-10 y	4.9-5.4
11-17 y	4.8-5.3

Pediatric data from *J Pediatr.* 1989;114: 952-956 and *Eur J Pediatr.* 1991;150: 464-467.

Interpretive Information

- Primary hyperparathyroidism
- Vitamin D intoxication
- Hypercalcemia of malignancy

- Hypoparathyroidism
- Secondary hyperparathyroidism

Clinical Background

Ionized calcium represents the concentration of free and biologically active calcium in blood. Calcium circulates in approximately equal free and bound fractions. Most (70%) of the bound calcium is bound to albumin. A portion of the non-protein-bound calcium is complexed to anions (bicarbonate, phosphate, and citrate). In the presence of an abnormal circulating albumin concentration, measurement of the plasma ionized calcium provides a more precise assessment of the biologically relevant calcium level. It permits more accurate diagnosis in hypocalcemic and hypercalcemic states.

Method

- Ion specific electrode
- Analytical sensitivity: 0.4 mg/dL

Specimen Requirements

2 mL refrigerated serum

0.6 mL minimum

Sample must be collected anaerobically in a gel-barrier tube. Allow to clot, then centrifuge with the cap on.

Calcium, Total 303X

Clinical Use

- Diagnose hypocalcemia or hypercalcemia
- Manage parathyroid disorders

Reference Range

	mg/dL
Adults	8.6-10.2
Children	
<1 mo	8.4-10.6
1-11 mo	8.7-10.5
1-3 y	8.5-10.6
4-19 y	8.9-10.4

Interpretive Information

- Primary hyperparathyroidism
- Hypercalcemia of malignancy
- Vitamin D intoxication

- Hypoparathyroidism
- Vitamin D deficiency
- Secondary hyperparathyroidism (renal disease)

Clinical Background

Blood calcium levels are precisely maintained, principally by vitamin D-regulated intestinal absorption of dietary calcium and parathyroid hormone (PTH) and vitamin D-mediated exchange of skeletal calcium stores. Hypercalcemia is most commonly due to malignancy and hyperparathyroidism and less often the result of vitamin D intoxication, granulomatous disorders, and several other rare conditions. Clinical manifestations of hypercalcemia can include neuropsychological, muscular, and cardiac effects. Hypocalcemia is most commonly caused by renal insufficiency with hyperphosphatemia, hypoparathyroidism, and vitamin D deficiency. Clinical manifestations of hypocalcemia can include paresthesia, muscle spasm, tetany, and seizures. The total calcium level, but not the ionized fraction, is low when the serum albumin concentration is decreased.

Simultaneous measurement of the total calcium and PTH concentrations, occasionally with vitamin D level assessment, permits differential diagnosis of most patients with an abnormal circulating calcium concentration.

Method

- Spectrophotometry
- Analytical sensitivity: 0.2 mg/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

Calcium, Urine

1635X
11216X

Clinical Use

- Assess metabolic disorders of calcium metabolism

Reference Range

Calcium (1635X)	mg/24-h
Men	50-300
Women	50-250
	mg/g creat
Men	30-210
Women	30-275
Creatinine (1635X)	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50
Calcium (11216X)*	mg/mg creat
1-11 mo	0.03-0.81
1 y	0.03-0.56
2 y	0.02-0.50
3-4 y	0.02-0.41
5-6 y	0.01-0.30
7-9 y	0.01-0.25
10-17 y	0.01-0.24
Creatinine (11216X)	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

*Matos V, et al. *J Pediatr.* 1997;131:252-257.

Interpretive Information

Increased
- Hyperparathyroidism
- Vitamin D intoxication
- Paget's disease
- Myeloma

Decreased
- Hypoparathyroidism
- Rickets
- Osteomalacia
- Hypocalcemia
- Hypocalciuric hypocalcemia

Clinical Background

Urinary calcium excretion is the major route of calcium elimination. Renal tubular calcium reabsorption is closely regulated to maintain serum calcium concentrations within a narrow physiologic range. The daily filtered load of calcium at the glomerulus approximates 9,000 mg/day in adults; only 1% to 3% of filtered calcium is excreted. Urinary calcium is bound in part by citrate, sulfate, and oxalate anions, and binding varies with pH, urine flow, and composition. It is difficult to ascertain whether changes in urine calcium relate to changes in filtered load, tubular reabsorption, or both. The usual urine calcium (mg/dL) to creatinine (mg/dL) ratio is 0.14 or less; values above 0.20 suggest hypercalciuria.

Method

- Spectrophotometry
- Analytical sensitivity: 0.2 mg/dL
- Creatinine concentration also reported

Specimen Requirements

Urine, 24-h (1635X)

10 mL refrigerated aliquot of a 24-h urine; 1 mL minimum

Collect urine in 25 mL of 6N HCl (preferred), 10 g boric acid, or 10 mL concentrated glacial acetic acid. Refrigerate during collection. Record 24-h volume on vial and request form.

Urine, 2nd void (11216X)

10 mL refrigerated aliquot of a 2nd void urine; 1 mL minimum

Adjust pH to <3.0 with 6N HCl prior to aliquoting.

Catecholamines, Fractionated, 24-Hour Urine

39627X

Clinical Use

- Diagnose catecholamine-secreting tumors

Reference Range

Epinephrine	µg/24-h
3-8 y	1-7
9-12 y	≤8
13-17 y	≤11
Adults	2-24
Norepinephrine	
3-8 y	5-41
9-12 y	5-50
13-17 y	12-88
Adults	15-100
Total N+E	
3-8 y	9-51
9-12 y	9-71
13-17 y	13-90
Adults	26-121
Dopamine	
3-8 y	80-378
9-12 y	51-474
13-17 y	51-645
Adults	52-480
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Pheochromocytoma
- Neuroblastoma
- Paragangliomas
- Stress

Clinical Background

Catecholamines are synthesized in chromaffin cells of the sympathetic nervous system: epinephrine by the adrenal medulla, and norepinephrine and dopamine by the adrenal medulla and postganglionic sympathetic neurons. Catecholamines are excreted in urine both intact and as metabolites (metanephrines and vanillylmandelic acid).

Elevated urinary catecholamine concentrations are detected in the majority of patients with pheochromocytoma. In patients with paroxysmal symptoms or hypertension, the sensitivity of testing can be increased by beginning urine collection immediately following an episode. The pattern of catecholamine secretion differs among tumor types: pheochromocytomas generally produce both norepinephrine and epinephrine; paragangliomas secrete norepinephrine; and neuroblastomas produce excessive dopamine.

Method

- High-performance liquid chromatography (HPLC), electrochemical detection
- Analytical sensitivity: 2 µg/L for epinephrine and norepinephrine; 10 µg/L for dopamine
- Creatinine concentration also reported

Specimen Requirements

10 mL room temperature aliquot of a 24-h urine; 4.5 mL minimum

Collect urine with 25 mL of 6N HCl. Record total volume on vial and request form.

It is preferable for the patient to be off medications for 3 d prior to collection; however, common antihypertensives cause minimal or no interference. Patient should avoid alcohol, coffee, tea, tobacco, and strenuous exercise prior to collection.

Catecholamines, Fractionated, Plasma

314X

Clinical Use

- Diagnose pheochromocytoma, paraganglioma, neuroblastoma, or postural hypotension

Reference Range

Adults	pg/mL Supine	pg/mL Upright
Epinephrine	<50	<95
Norepinephrine	112-658	217-1109
Dopamine	<10	<20
Total (N+E)	123-671	242-1125
Children (3-15 y)	Supine	
Epinephrine	≤464	
Norephinephrine	≤1251	
Dopamine	<60	

Due to stress, plasma catecholamine levels are generally unreliable in infants and small children. Urinary catecholamine assays are more reliable.

Pediatric data from *J Chromatogr*. 1993; 617:304-307.

Interpretive Information

- Pheochromocytoma
- Ganglioneuroma
- Neuroblastoma
- Severe stress
- Hypoglycemia

- Postural hypotension
- Shy-Drager syndrome
- Familial dysautonomia

Clinical Background

Catecholamines are synthesized in chromaffin cells of the sympathetic nervous system: epinephrine by the adrenal medulla, and norepinephrine and dopamine by the adrenal medulla and postganglionic sympathetic neurons. Catecholamines circulate in plasma in both free and bound forms complexed to albumin, globulins, and lipoproteins. Assays measure the free and protein-bound, but not conjugated fractions.

Plasma catecholamines exhibit considerable physiological variability, increasing with pain and psychological stress. Consequently, samples should be obtained from patients in the supine position and basal state, ideally by using an indwelling venous catheter at least 30 minutes after venipuncture. Specificity of elevated plasma catecholamine measurements may be increased by suppression and/or stimulation testing (see Dynamic Test section). Certain medications may increase the catecholamine measurement either by assay interference (eg, methyldopa, isoproterenol) or by increasing endogenous catecholamine release (eg, nitrates, minoxidil, hydralazine); cigarette smoking and caffeine ingestion may do so as well.

Method

- HPLC, electrochemical detection
- Analytical sensitivity: 20 pg/mL for epinephrine and norepinephrine; 10 pg/mL for dopamine

Specimen Requirements

4 mL frozen sodium heparin plasma

2.5 mL minimum

Collect in pre-chilled vacutainer. Centrifuge in refrigerated centrifuge within 30 minutes of collection. Separate plasma and freeze immediately.

Patient should avoid alcohol, coffee, tea, tobacco, and strenuous exercise prior to collection. Overnight fasting is required.

Catecholamines, Fractionated, Random Urine

5244X

Clinical Use

- Diagnose catecholamine-secreting tumors

Reference Range

	E µg/g creat	*N* µg/g creat
Birth to 6 mo	2-45	12-286
7-11 mo	5-45	19-250
1-2 y	1-49	25-210
3-8 y	4-32	20-108
9-12 y	1-15	20-73
13-17 y	1-10	15-58
Adults	2-16	7-65

	Total N+E µg/g creat	*D* µg/g creat
Birth to 6 mo	24-322	107-2180
7-11 mo	10-295	96-2441
1-2 y	30-263	86-1861
3-8 y	30-130	295-1123
9-12 y	25-90	164-744
13-17 y	20-70	156-551
Adults	9-74	40-390

Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

E, epinephrine; N, norephinephrine; D, dopamine.

Interpretive Information

- Pheochromocytoma
- Neuroblastoma
- Paragangliomas
- Stress

Clinical Background

Catecholamines are synthesized in chromaffin cells of the sympathetic nervous system: epinephrine by the adrenal medulla, and norepinephrine and dopamine by the adrenal medulla and postganglionic sympathetic neurons. Catecholamines are excreted in urine both intact and as metabolites (metanephrines and vanillylmandelic acid).

Elevated urinary catecholamine concentrations are detected in the majority of patients with pheochromocytoma. In patients with paroxysmal symptoms or hypertension, the sensitivity of testing can be increased by beginning urine collection immediately following an episode. The pattern of catecholamine secretion differs among tumor types: pheochromocytomas generally produce both norepinephrine and epinephrine; paragangliomas secrete norepinephrine; and neuroblastomas produce excessive dopamine.

Method

- HPLC, electrochemical detection
- Analytical sensitivity: 2 µg/L for epinephrine and norepinephrine; 10 µg/L for dopamine
- Creatinine concentration also reported

Specimen Requirements

10 mL room temperature urine

4 mL minimum

Add 25 mL of 6N HCl to maintain a pH below 3.

It is preferable for the patient to be off medications for 3 d prior to collection; however, common antihypertensives cause minimal or no interference. Patient should avoid alcohol, coffee, tea, tobacco, and strenuous exercise prior to collection.

Central Diabetes Insipidus (CDI) Mutations 15035X

Clinical Use

- Differentiate inherited CDI from acquired CDI
- Screen for CDI carrier status in at-risk individuals

Reference Range

Negative (no mutations detected)

Interpretive Information

Mutation present

- Central diabetes insipidus (affected or carrier)

Clinical Background

CDI is an acquired or autosomal dominant inherited disorder characterized by polyuria, polydipsia, a low urinary specific gravity, and high risk of severe dehydration. Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is absent. CDI stems from the degeneration or destruction of cells in the posterior pituitary, the site of AVP production. Thus, it is also referred to as pituitary, neurohypophyseal, or neurogenic diabetes insipidus. The disorder typically presents in infancy or early childhood, although late-onset cases have been reported.

Although rare, inherited CDI can be caused by mutations in the AVP gene on chromosome 20. Prepro-AVP, the initial protein product of the AVP gene, undergoes several post-translational steps to yield AVP, neurophysin, and glycoprotein. When mutations in the AVP gene are present, cytotoxic products that lead to destruction of the secretory neurons are generated.

Since more than 30 relevant mutations have been identified, gene sequencing is the method of choice for diagnosis of the inherited form. After identification of a mutation in an affected individual, genetic testing can be used to evaluate other family members.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Analytical specificity: mutations in 3 exons of the AVP gene

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube.

Chromogranin A 34468X

Clinical Use

- Assess peptide-secreting endocrine neoplasms
- Assess multiple endocrine neoplasia

Reference Range

Adults	≤36.4 ng/mL

Interpretive Information

- Anterior pituitary adenoma
- Parathyroid adenoma
- Medullary thyroid carcinoma
- Carcinoid tumor
- Pancreatic islet cell tumor
- Small cell lung carcinoma

Clinical Background

Chromogranin A (CGA) is the major protein within the catecholamine storage vesicles (chromaffin granules) of the adrenal medulla. When catecholamines are released by exocytosis from normal adrenal medulla or pheochromocytoma cells, all vesicle contents are co-released, including CGA. CGA has also been shown to be co-released with polypeptide hormones from the following endocrine tissues: pancreatic islet cells, enteroendocrine cells, parathyroid chief cells, thyroid parafollicular C cells, and anterior pituitary cells. CGA concentrations may be elevated in patients with various endocrine neoplasms, eg, anterior pituitary adenoma, parathyroid adenoma, medullary thyroid carcinoma, carcinoid tumor, and pancreatic islet cell tumor. In contrast, patients with non-endocrine neoplasia or benign endocrine disease have normal plasma CGA concentrations. Serum CGA also has been reported to be elevated in patients with small cell lung carcinoma.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 1.5 ng/mL

Specimen Requirements

0.8 mL refrigerated serum (no additive red top tube); 0.3 mL minimum

SST red top unacceptable

Collagen Cross-Linked N-Telopeptide (NTx), Urine

36167X
36421X

Clinical Use

- Monitor therapeutic response in patients with metabolic bone disorders
- Predict future bone mineral density (BMD)
- Predict therapeutic response prior to initiation of antiresorptive therapy
- Detect bone metastasis in patients with various malignancies

Reference Range

Men	Units*
2nd AM urine	
18-29 y	12-99
30-59 y	9-60
24-h urine	5-87
Women, premenopausal	
2nd AM urine	4-64
24-h urine	5-79

Test results within the premenopausal reference range do not rule out osteoporosis nor the need for therapy.

*nmol BCE/mmol creat; BCE, bone collagen equivalent.

Interpretive Information

↑
- Osteoporosis
- Osteopenia
- Celiac disease
- Paget's disease
- Primary hyperthyroidism
- Rheumatoid arthritis
- Growth hormone deficiency (non-adult onset)

↓
- Therapeutic response (ie, decrease relative to baseline)

Clinical Background

Healthy levels of bone mineral density (BMD) are maintained by a balance between bone resorption and bone formation. N-telopeptide (NTx), the amino-terminal cross-linked peptide of type I collagen, is released during bone resorption and has been correlated with BMD T-scores. Multiple studies have shown that NTx not only correlates inversely with BMD response to therapy, but also is an early marker or predictor of BMD response. Thus, therapeutic response can be determined within 3 to 6 months of therapy rather than 1 to 2 years. Studies have also demonstrated that elevated pretreatment NTx values predict positive response to therapies such as hormone replacement therapy in postmenopausal women. In patients with malignancies, elevated levels of NTx may indicate bone metastases.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 10 nmol BCE/L

Specimen Requirements

Urine, 2nd morning void (36167X)
Urine, 24-h collection (36421X)

2 mL refrigerated aliquot of urine
1 mL minimum
Do not use preservatives.

Record total 24-h volume on the vial and request form.

Collagen Type 1 C-Telopeptide (CTx) 17406X

Clinical Use

- Monitor therapeutic response in patients with metabolic bone disorders
- Predict future bone mineral density (BMD)
- Predict therapeutic response prior to initiation of antiresorptive therapy
- Detect bone metastasis in patients with various malignancies

Reference Range

Men	pg/mL
18-29 y	87-1200
30-39 y	70-780
40-49 y	60-700
50-68 y	87-345
Women	
18-29 y	64-640
30-39 y	60-650
40-49 y	40-465

Interpretive Information

↑
- Osteoporosis
- Osteopenia
- Celiac disease
- Paget's disease
- Primary hyperthyroidism
- Rheumatoid arthritis
- Growth hormone deficiency (non-adult onset)

↓
- Therapeutic response (ie, decrease relative to baseline)

Clinical Background

Healthy levels of bone mineral density (BMD) are maintained by a balance between bone resorption and bone formation. C-telopeptide (CTx), a cross-linked peptide of type I collagen, is released during bone resorption and has been correlated with BMD T-scores. CTx correlates inversely with BMD response to therapy and is an early marker or predictor of BMD response. Thus, therapeutic response can be determined within 3 to 6 months of therapy rather than 1 to 2 years. Studies have demonstrated that elevated pretreatment CTx values predict positive response to therapies such as hormone replacement therapy in postmenopausal women. In patients with malignancies, elevated levels of CTx may indicate bone metastases.

Method

- Electrochemiluminescent immunoassay (ECLIA)
- Analytical sensitivity: 30 pg/mL

Specimen Requirements

1 mL frozen serum (no additive red top tube); 0.5 mL minimum

Collect sample between 8 and 10 am after an 8 to 12 hour fast.

Corticosterone, LC/MS/MS 6547X

Clinical Use

- Assess adrenal gland function
- Marker for $P450_{c17}$ enzyme deficiency

Reference Range

	ng/dL	ng/dL
Adults		
8-10 AM		59-1293
4-6 PM		≤386
Children		
Premature infants (31-35 wks)*		≤235
Term infants*		≤170
	Male	Female
<1 y	78-1750	89-1200
1-4 y	120-1290	160-2040
5-9 y	≤677	≤677
10-13 y	≤1420	≤1420
14-17 y	≤1238	≤1238
Tanner stages		
II-III	115-1220	110-600
IV-V	165-840	160-390

**J Clin Endocrinol Metab.* 1992;75:1491-1496.

Interpretive Information

- Adrenal adenoma
- Cushing's disease
- $P450_{c17}$ (17-hydroxylase) deficiency
- $P450_{c11ase}$ (aldosterone synthetase) deficiency

- Addison's disease
- Adrenal hypoplasia
- ACTH deficiency

Clinical Background

Corticosterone is an intermediate in the adrenal corticosteroid biosynthetic pathway. It is the substrate for further 18-hydroxylation and dehydrogenation to aldosterone. Corticosterone synthesis from 11-deoxycorticosterone (DOC) is catalyzed by $P450_{c11}$. Measurement is useful in Cushing's syndrome when exogenous steroid use is suspected. Rare cases of corticosterone-secreting adenomas have been reported.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 20.0 ng/dL

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable

Corticotropin Releasing Hormone (CRH) 36589X

Clinical Use

- Differential diagnosis of Cushing's syndrome

Reference Range

	pg/mL
Men	≤34
Women	
Nonpregnant	≤34
1st trimester pregnancy	≤40
2nd trimester pregnancy	≤153
3rd trimester pregnancy	≤847
Children	
Cord Blood	≤338

Pregnancy and cord blood reference ranges from *J Clin Endocrinol Metab.* 1986;63:1199-1203.

Interpretive Information

- ↑ Cushing's syndrome (ectopic CRH)
- ↑ Pregnancy
- ↑ Fetus
- ↓ Cushing's syndrome (adrenal)

Clinical Background

CRH, a 41-amino acid peptide normally produced in the hypothalamus, stimulates the release of ACTH. Normal blood levels of CRH are low, but CRH is also produced by the placenta, and high levels are measured during the second and third trimesters of pregnancy. CRH levels also are increased in Cushing's syndrome due to ectopic production.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 28 pg/mL
- Analytical specificity: no cross-reactivity with ACTH, LH releasing hormone, PACAP-38, arginine vasopressin, urocortin, urocorin, or BNP

Specimen Requirements

3.1 mL refrigerated plasma

1.1 mL minimum

Collect blood in special Nichols Institute PTH-RP and Releasing Factor collection tube.

Centrifuge immediately in refrigerated centrifuge, remove plasma, and refrigerate.

Cortisol-Binding Globulin (CBG) 37371X

Clinical Use

- Useful in evaluating unexplained cortisol concentrations

Reference Range

	mg/L
Adults	19-45
Children	
Preterm infants (<8 d)	6-26

Pediatric data from *Pediatr Res.*1994;37: 112-116.

Interpretive Information

Increased

- Pregnancy
- Estrogen therapy
- Chronic, active hepatitis
- Inherited abnormality

Decreased

- Hyperinsulinemic states
- Nephrotic syndrome
- Severe liver disease
- Malnutrition
- Newborn
- Inherited abnormality

Clinical Background

Cortisol-binding globulin (CBG) is a serum alpha-2-globulin-binding protein with high affinity and limited capacity for cortisol. It binds most (±92%) of the serum cortisol; the remaining cortisol (±8%) circulates in a free form or bound to albumin.

CBG is synthesized in the liver. Circulating levels increase in pregnancy or after administration of estrogen or oral contraceptives. Insulin may inhibit synthesis, a fact that might explain the low CBG levels observed in hyperinsulinemic states (eg, diabetes, obesity, PCO). CBG levels are also reduced in nephrotic syndrome, starvation, or severe chronic liver disease.

The CBG assay is helpful in assessing unexpected values of blood cortisol. Other assays useful in diagnosis and therapy of cortisol abnormalities include plasma cortisol, ACTH, β-lipotropin, urinary cortisol (by HPLC), or free cortisol in plasma or serum.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 0.1 mg/L

Specimen Requirements

1 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Cortisol, Free and Cortisone, 24-Hour Urine

37355X

Clinical Use

- Diagnose Cushing's syndrome
- Differentiate between exogenous and endogenous Cushing's syndrome
- Marker for apparent mineralocorticoid excess (AME) syndrome

Reference Range

Cortisol, Free	µg/24-h
Adults	4.0-50.0
Children	
1-4.9 y	0.9-8.2
5-9.9 y	1.0-30.0
10-13.9 y	1.0-45.0
14-17.9 y	3.0-55.0
Cortisone	µg/24-h
Adults	23-195
Children	
1-4.9 y	5-40
5-9.9 y	9-150
10-13.9 y	11-145
14-17.9 y	1-166
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Cushing's syndrome

↓
- Exogenous Cushing's syndrome
- Adrenal insufficiency

Clinical Background

The differential diagnosis of Cushing's syndrome has been based on ACTH and cortisol assays in urine or serum. Recent data have shown utility for combined urinary free cortisol and cortisone measurements by high performance liquid chromatography (HPLC). Using HPLC, the diagnostic sensitivity for Cushing's syndrome is 93%, an increase of 17% over cortisol and 7% over cortisone. Both cortisol and cortisone levels are elevated in patients with pituitary, ectopic, and adrenal Cushing's syndrome, while both cortisol and cortisone levels are decreased in patients receiving exogenous glucocorticoid (eg, prednisolone). Additionally, the measurement of cortisol and cortisone can be used for the diagnosis of apparent mineralocorticoid excess (AME) in which cortisone formation is reduced, allowing cortisol to act as a mineralocorticoid.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 µg/L
- Analytical specificity: high concentrations of 20β-DHE/DHF decrease results; high concentrations of prednisolone interfere with measurement.
- Creatinine concentration also reported

Specimen Requirements

10 mL refrigerated aliquot of a 24-h urine; 2.6 mL minimum

Collect urine with 10 g of boric acid or keep refrigerated during collection. Record 24-h volume on vial and request form.

Cortisol, Free, 24-Hour Urine 11280X

Clinical Use

- Screen for Cushing's syndrome and adrenal insufficiency

Reference Range

Cortisol, Free	µg/24-h
Adults	4-50
Children	
1-4 y	0.9-8.2
5-9 y	1.0-30.0
10-13 y	1.0-45.0
14-17 y	3.0-55.0
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

- ↑ Adrenal hyperfunction, ie, Cushing's disease
- ↓ Adrenal insufficiency

Clinical Background

Cortisol primarily circulates bound to cortisol-binding globulin (CBG). Only the unbound (free) cortisol appears to be biologically active. The free fraction is 5% to 8% of total cortisol in normal individuals.

Since the cortisol binding capacity of blood proteins is limited, adrenal hyperfunction (Cushing's syndrome) is invariably associated with a many-fold increase in urinary cortisol levels. Urinary free cortisol is not substantially increased in pregnancy, obesity, diabetes, or hyperthyroidism, entities that in the past could represent a diagnostic dilemma when older assays of cortisol or metabolites were used.

Exogenous Cushing's syndrome due to surreptitious use of glucocorticoid can now be diagnosed by finding low cortisol and presence of synthetic steroids in the urine.

Urinary cortisol is the best screening test for Cushing's syndrome and can be used to rule out Addison's disease.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 µg/L
- Creatinine concentration also reported

Specimen Requirements

2 mL frozen aliquot of a 24-h urine

0.5 mL minimum

No preservative (preferred)

25 mL 6N HCL acceptable

10 g boric acid acceptable

10 mL concentrated glacial acetic acid acceptable

Record 24-h volume on vial and request form.

Cortisol, Free, Saliva 16164X

Clinical Use

- Screen for Cushing's syndrome
- Assess diurnal changes in free cortisol

Reference Range

See report.

Interpretive Information

	Salivary Cortisol	Total Cortisol	CBG
Pregnancy	N	↑	↑
Oral contraceptives	N	↑	↑
Estrogen therapy	N	↑	↑
Chronic liver disease	N	↓	↓
Starvation	N	↓	↓
Nephrotic syndrome	N	↓	↓
Adrenal insufficiency	↓	↓	N
Cushing's syndrome	↑	↑	N

CBG, cortisol binding globulin; N, normal.

Clinical Background

Cortisol (hydrocortisone or compound F) is a glucocorticoid secreted by the adrenal cortex after adrenocorticotropic hormone (ACTH) stimulation. More than 90% of circulating cortisol is bound to cortisol- binding globulin (CBG or transcortin). CBG levels are increased in pregnancy, estrogen therapy, and oral contraceptives, and decreased in nephrotic syndrome, starvation, and chronic liver disease. Free cortisol diffuses through the acinar cells of the salivary glands. Salivary levels are about two-thirds that of serum. Measurements of salivary cortisol correlate with serum levels within the physiological range, but the ratio of serum to salivary free cortisol increases at higher levels. The correlation (r) between salivary free cortisol and urinary free cortisol is 0.63.

The measurement of salivary cortisol is useful as a screening test for Cushing's syndrome. Cortisol is secreted episodically, but there is a circadian rhythm with peak levels at 7-8 AM and nadir values soon after midnight. In addition to increased cortisol secretion in patients with Cushing's syndrome, disruption of the circadian rhythm is common, ranging from subtle alterations to complete disruption. Measurement of morning and late night salivary cortisol concentrations provides a stress-free screening approach in children and adults. Salivary cortisol measurements also have been used to assess the response to overnight dexamethasone.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Provides best measurement of biologically active cortisol
- Analytical sensitivity: 0.1 µg/dL

Specimen Requirements

0.5 mL refrigerated saliva

0.2 mL minimum

Patient should refrain from food and cigarettes for 1 hour prior to sample collection. Collect using Salivette tube (Sarstedt) 10 minutes after rinsing mouth with water.

Cortisol, Free, Serum 36423X

Clinical Use

- Determine glucocorticoid status

Reference Range

See report

Interpretive Information

	Free Cortisol	Total Cortisol	CBG
Pregnancy	N	↑	↑
Oral contraceptives	N	↑	↑
Estrogen therapy	N	↑	↑
Chronic liver disease	N	↓	↓
Starvation	N	↓	↓
Nephrotic syndrome	N	↓	↓
Adrenal insufficiency	↓	↓	N
Cushing's syndrome	↑	↑	N

CBG, cortisol binding globulin; N, normal.

Clinical Background

Cortisol (hydrocortisone or compound F) is a glucocorticoid secreted by the adrenal cortex after adrenocorticotropic hormone (ACTH) stimulation. More than 90% of circulating cortisol is bound to cortisol-binding globulin (CBG or transcortin). CBG levels are increased in pregnancy, estrogen therapy, and oral contraceptives, and decreased in nephrotic syndrome, starvation, and chronic liver disease. Thus, total cortisol levels can be affected by these conditions.

Free cortisol, however, is the physiologically active form. Free cortisol levels change independently and accurately reflect the clinical status; thus serum free cortisol is the most reliable test for glucocorticoid status. It is markedly elevated in Cushing's syndrome and falls to almost undetectable levels after dexamethasone (1 mg overnight) in normal individuals.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Provides best measurement of biologically active cortisol
- Analytical sensitivity: 0.1 µg/dL

Specimen Requirements

2 mL refrigerated serum

0.7 mL minimum

No additive red top tube preferred

SST red top acceptable

Cortisol, Total, LC/MS/MS 11281X

Clinical Use

- Direct assessment of adrenal function

Reference Range

Adults	µg/dL	µg/dL
8-10 AM		5-21
4-6 PM		2-14
Post ACTH stimulation		
Peak		>20
Peak after IM injection		>16
Children		
Premature infants (31-35 wk)		≤15
Term infants (3 d)		≤14
<1 y		NA
1-17 y		2-17
ACTH stimulation	Baseline	60 min
1-12 mo	3-23	32-60
1-5 y	6-25	22-40
6-12 y	3-15	17-28
Tanner II-III		
Males	4-13	15-45
Females	4-16	16-32
Tanner IV-V		
Males	5-15	18-27
Females	6-15	18-35

Pediatric data from *J Clin Endocrinol Metab.* 1991;73:674-686 and *J Clin Endocrinol Metab.* 1989;69:1133-1136.

Interpretive Information

↑
- Cushing's syndrome
- Ectopic ACTH syndrome
- Ectopic CRH syndrome
- Adrenal adenoma or carcinoma
- Adrenal micronodular dysplasia
- Adrenal macronodular hyperplasia
- Stress

↓
- Addison's disease
- Pituitary dysfunction (decreased production of ACTH)

Diurnal rhythmicity increased by systemic disease and other stress

Clinical Background

Cortisol is the major glucocorticoid secreted by the adrenal gland. Secretion is regulated by ACTH in a diurnal fashion. ACTH secretion peaks in the early morning hours, stimulating a morning peak of serum cortisol concentration. Cortisol, therefore, is best measured in the morning (8 AM) when evaluating for possible adrenal insufficiency and best measured in the afternoon or evening (4-11 PM) to differentiate normal and Cushing's syndrome subjects. Baseline and post-dexamethasone suppression values may be useful in differential diagnosis. ACTH-stimulated values also may be useful. Combined measurements of serum cortisol and ACTH provide differential diagnostic discrimination in most cases.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 0.1 µg/dL
- Analytical specificity: high concentrations of 20β-DHE/DHF decrease results; high concentrations of prednisolone interfere with measurement.

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.2 mL minimum

Specify time of day specimen was collected.

Cortisone, 24-Hour Urine 37554X

Clinical Use

- Evaluate adrenal function
- Marker for AME
- Additional marker for exogenous steroid use

Reference Range

Cortisone	µg/24-h
Adults	23-195
Children	
1-4.9 y	5-40
5-9.9 y	9-150
10-13.9 y	11-145
14-17.9 y	1-166
Creatinine	**g/24-h**
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Cushing's syndrome

↓
- Adrenal insufficiency
- Exogenous Cushing's syndrome (prednisone)
- AME

Clinical Background

Cortisol, the active glucocorticoid produced by the adrenal gland, is inactivated to cortisone in peripheral tissues. Excretion of both cortisol and cortisone is increased in patients with Cushing's syndrome but reduced in patients with adrenal insufficiency and in those receiving exogenous glucocorticoid (eg, prednisone). In patients with apparent mineralocorticoid excess (AME), cortisone formation is reduced, allowing cortisol to act as a mineralocorticoid. Patients with AME have reduced cortisone and aldosterone excretion, while urinary free cortisol is normal or increased.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1.0 µg/L
- Analytical specificity: high concentrations of 20β-DHE/DHF decrease results; high concentrations of prednisolone interfere with measurement.
- Creatinine concentration also reported

Specimen Requirements

10 mL refrigerated aliquot of a 24-h urine; 2.1 mL minimum

Collect urine with 10 g of boric acid or keep refrigerated during collection. Record 24-h volume on vial and request form.

Cortisone, Serum 37098X

Clinical Use

- Evaluate adrenal function
- Marker for AME
- Additional marker for exogenous steroid use

Reference Range

	µg/dL
Adults	
AM	1.2-3.5
PM	0.6-2.8
Children (AM)	
Full-term infants (birth)	2.6-15.6
7 d	0.3-4.5
2 wk-3 mo	0.9-5.4
3 mo-1 y	0.7-4.6
1-17 y	0.6-3.0

Pediatric data from Sippell WG, et al. *Pediatr Res.* 1980;14:39-46.

Interpretive Information

- Cushing's syndrome

- Adrenal insufficiency
- Exogenous Cushing's (prednisone)
- AME

Clinical Background

Cortisol, the active glucocorticoid produced by the adrenal gland, is inactivated to cortisone in peripheral tissues. Both cortisol and cortisone levels are increased in patients with Cushing's syndrome but reduced in patients receiving exogenous glucocorticoid (eg, prednisone). In apparent mineralocorticoid excess (AME), cortisone formation is reduced, allowing cortisol to act as a mineralocorticoid. Patients with AME have reduced cortisone and aldosterone levels but normal cortisol levels.

Licorice ingestion can inhibit conversion of cortisol to cortisone, thereby decreasing circulating cortisone levels.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 0.1 µg/dL
- Analytical specificity: high concentrations of 20β-DHE/DHF decrease results; high concentrations of prednisolone interfere with measurement.

Specimen Requirements

1 mL refrigerated serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting preferred

Specify time of day specimen was collected.

C-Peptide, Serum or Urine

372X. 4643X, 11182X

Clinical Use

- Assess pancreatic islet cell function

Reference Range

Serum (372X)

C-Peptide	ng/mL
Adults	0.8-3.1

Urine 24-h (4643X)

C-Peptide	µg/g creat
Adults	10.8-107.9
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Urine 2nd void (11182X)

C-Peptide	µg/g creat
Men	5-79
Women	7-142
Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

Interpretive Information

↑
- Insulinoma
- Type 2 diabetes

↓
- Type 1 diabetes
- Exogenous insulin administration

Clinical Background

C-peptide is a 31-amino acid that connects insulin's 2 peptide chains; it has no known biologic activity. Because of differences in half-life and hepatic clearance, peripheral blood levels of C-peptide and insulin are no longer equimolar but still remain highly correlated under resting and perturbatory conditions.

Measurement of C-peptide is useful for distinguishing 1) insulin-secreting tumors (insulinoma) from exogenous insulin administration as a cause of hypoglycemia (commercial insulin does not contain C-peptide); and 2) type 1 from type 2 diabetes mellitus (type 2 is associated with abundant C-peptide secretion; type 1 has little or no C-peptide secretion).

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.5 ng/mL (serum) and 0.5 µg/mL (urine)

Specimen Requirements

Serum (372X)

2 mL frozen serum

0.4 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is required.

Urine 24-h (4643X)

2 mL frozen aliquot of a 24-h urine

0.5 mL minimum

Do not use a preservative.

Record 24-h volume on vial and request form.

Urine 2nd Void (11182X)

2 mL frozen aliquot of a 2nd void urine; 1 mL minimum

Cyclic Adenosine Monophosphate (Cyclic AMP) 37555X, 242X, 225X

Clinical Use

- Diagnose hyperparathyroidism
- Diagnose pseudohypoparathyroidism subtypes
- Assess severity of hypoparathyroidism

Reference Range

Nephrogenous (37555X)
1.4-5.0 nmol/dL

Plasma (242X, 37555X)
3.9-13.1 nmol/L

Random urine (225X, 37555X)
0.8-7.5 µmol/L

Creatinine, Plasma (37555X)

	mg/dL
Adults	0.6-1.4
Children, full-term infants*	
0-2 d	0.7-1.4
3-5 d	0.4-1.1
6-7 d	0.3-0.9
1 wk-1 mo	0.3-0.8
1 mo-1 y	0.2-0.7
2-9 y	0.3-0.7
10-17 y	0.5-1.0

***Creatinine, random urine* (*37555X*)**

	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20–370
Females	20-320

*Values for children <2 y from Savory DJ, *Ann Clin Biochem.* 1990;27:99-101.

Interpretive Information

↑
- Hyperparathyroidism
- Hypercalcemia of malignancy

↓
- Hypoparathyroidism
- Type I pseudohypoparathyroidism
- Renal insufficiency

Clinical Background

3´, 5´ cyclic adenosine monophosphate (cAMP) is an intracellular second messenger produced by the action of adenyl cyclase upon ATP. Significant amounts of intracellular cAMP appear in plasma with a diurnal rhythm peak at 1200 hours and a nadir in late evening. Approximately half of urinary cAMP is filtered from plasma while the other half is derived from kidney tissue. cAMP in the kidney is generated following parathyroid hormone (PTH) stimulation of renal PTH receptors.

Urinary cAMP is generally elevated in patients with hyperparathyroidism. It may also be increased in cancer patients with hypercalcemia. Some patients with pseudohypoparathyroidism, a condition characterized by decreased renal responsiveness to parathyroid hormone, fail to respond with a rise in urinary cAMP when exogenous PTH is given. Measurement of nephrogenous cAMP may still be used on rare occasions to diagnose subtypes of pseudohypoparathyroidism.

Method

- Extraction, radioimmunoassay

Specimen Requirements

Nephrogenous (37555X)
25 mL frozen urine and 5 mL frozen EDTA plasma; 2.5 mL and 1.2 mL minimum, respectively

Collect urine with 2.0 mL of 6N HCl to maintain pH below 3.

Plasma (242X)
3.1 mL frozen EDTA plasma
1 mL minimum

Random urine (225X)
1 mL frozen urine
0.2 mL minimum

Collect urine with 2.0 mL of 6N HCl to maintain pH below 3.

Cystatin C 10570X

Clinical Use

- Assess glomerular filtration rate in type 1 and 2 diabetes mellitus

Reference Range

Children & Adults	0.5-1.0 mg/L

Interpretive Information

- Impaired glomerular filtration

Clinical Background

Cystatin C is a low molecular weight (13,359 Da) protein belonging to the superfamily of cystine protease inhibitors. It is produced by all nucleated cells at a reasonably constant rate; production is minimally affected by diet, inflammatory states, lean body mass, or circadian rhythm. Like creatinine, cystatin C is freely filtered by the glomeruli. It is reabsorbed and metabolized by renal tubular cells and does not appear in urine. Tan et al (*Diabetes Care*. 2002;25:2004-2009) showed serum cystatin C has good correlation with creatinine clearance ($p = 0.74$) and iohexol clearance ($p = -0.80$) and is a useful marker of renal dysfunction in diabetic patients with minimal tubular reabsorption. Because creatinine production is relatively variable, cystatin C has been proposed as a better marker of glomerular filtration.

Method

- Nephelometry

Specimen Requirements

1.0 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is required.

Deoxycorticosterone (DOC) 6559X

Clinical Use

- Diagnose adrenal enzyme deficiencies
- Assess adrenal functional disorders

Reference Range

		ng/dL
Men		3.5-11.5
Women		
Follicular phase		1.5-8.5
Luteal phase		3.5-13.0
Pregnancy		
First trimester		5-25
Second trimester		10-75
Third trimester		30-110
ACTH stimulation	Baseline ng/dL	60 min ng/dL
Men and premenopausal women (follicular phase)		14-33
<1 y	7-57	20-160
1-5 y	4-49	26-140
6-12 y		
Males	9-34	33-140
Females	2-13	19-61
Tanner II-III		
Males	4-30	12-74
Females	2-12	13-63
Tanner IV-V		
Males	5-14	19-46
Females	5-10	23-40

Pediatric data from *J Clin Endocrinol Metab.* 1991;73:674-686.

Interpretive Information

↑
- Congenital adrenal hyperplasia (P-450$_{c11}$ deficiency)
- P-450$_{c17}$ deficiency
- P-450$_{c11}$ase (aldosterone synthetase) deficiency
- Cushing's syndrome
- Primary aldosteronism
- Low renin essential hypertension
- Adrenal carcinoma (some cases)

↓
- Adrenal insufficiency
- Adrenal hypoplasia

Clinical Background

Deoxycorticosterone (DOC) is a weak mineralocorticoid derived from 21-hydroxylation of progesterone in the adrenal cortex. DOC is a precursor of corticosterone and aldosterone and, like cortisol, is under the primary control of ACTH. DOC can be used therapeutically as a replacement mineralocorticoid in patients with primary adrenal insufficiency or isolated hypoaldosteronism.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 1 ng/dL

Specimen Requirements

3 mL refrigerated serum

1.1 mL minimum

No additive red top preferred

SST red top acceptable

11-Deoxycortisol, LC/MS/MS 30543X

Clinical Use

- Marker for adrenal P-450$_{c11}$ (11β-hydroxylase) enzyme deficiency
- Assess pituitary-adrenal reserve with the metyrapone test

Reference Range

	ng/dL
Men	
18-29 y	≤119
30-39 y	≤135
40-49 y	≤76
50-59 y	≤42
Women	
18-29 y	≤107
30-39 y	≤51
40-49 y	≤62
50-66 y	≤37
Children	
Premature infants (31-35 wk)*	≤235
Term infants*	≤170
1-12 mo	10-200
1-4 y	7-210
5-9 y	≤122
10-13 y	≤245
14-17 y	≤302
Tanner II-III	
Males	11-150
Females	15-130
Tanner IV-V	
Males	14-120
Females	17-120

*Data from *J Clin Endocrinol Metab.* 1991; 73:674-686 and *J Clin Endocrinol Metab.* 1989; 69:1133-1136.

Marked elevation of 21-deoxycortisol, as may occur in 21-hydroxylase deficiency, can increase apparent levels of 11-deoxycortisol.

Interpretive Information

- ↑ Congenital adrenal hyperplasia (P-450$_{c11}$ deficiency)
- ↑ After metyrapone in normal subjects
- ↓ Adrenal insufficiency

Clinical Background

Cortisol is formed in the adrenal gland by the enzymatic action of 11β-hydroxylase (P-450$_{c11}$) on 11-deoxycortisol. Deficiency of this enzyme causes congenital adrenal hyperplasia (CAH) characterized by hypertension and virilization. ACTH, 11-deoxycortisol, and androgen levels are all increased. This defect accounts for about 5% of CAH cases.

The drug metyrapone blocks 11β-hydroxylase, which in normal individuals results in increased ACTH and 11-deoxycortisol production. Thus, the drug is used to test adrenal and pituitary reserve. In patients with primary and secondary adrenal insufficiency, there is little or no increase in 11-deoxycortisol in response to metyrapone administration. In patients with Cushing's syndrome caused by ectopic ACTH or adrenal tumors, metyrapone does not change ACTH or 11-deoxycortisol levels (see Dynamic Test section). Measurements of cortisol and ACTH have generally replaced the metyrapone test.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 20.0 ng/dL

Specimen Requirements

1 mL frozen serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable.

An early-morning specimen is preferred.

Deoxypyridinoline (DPD, DPYD), Free 29497X

Clinical Use

- Assess rate of bone collagen degradation
- Monitor therapy

Reference Range

DPD, Free	
Adults (20-58 y)*	nmol/mmol creat
Men	2.1-8.1
Women	3.3-13.5
Children	
3-9 y	13.0-41.9
10-13 y	11.8-35.1
14-19 y	6.8-27.7
Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

*Test results within the premenopausal reference range do not rule out osteoporosis or the need for therapy.

Interpretive Information

↑
- Osteoporosis
- Primary hyperparathyroidism
- Paget's disease

↓
- Relative to baseline indicates therapeutic response

Clinical Background

Collagen fibers are linked together by interchain molecules referred to as pyridinium cross-links. Collagen pyridinium cross-links have been identified in all connective tissue except skin. As collagen is broken down by collagenase, small breakdown products are excreted in urine with the attached cross-links, including pyridinoline and deoxypyridinoline (DPD). Bone collagen is constantly turning over and is rich in DPD. Thus, urine DPD is a useful marker for bone matrix degradation and bone resorption.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 nmol/L
- Creatinine concentration also reported

Specimen Requirements

3 mL refrigerated urine

1 mL minimum

2nd morning void preferred, prior to 10 AM.

Do not use preservatives.

Dexamethasone 29391X

Clinical Use

- Monitor therapy or compliance
- Evaluate hypothalamic-pituitary-adrenal axis

Reference Range

	ng/dL
Baseline	<20
8-10 AM, after 1 mg overnight dexamethasone	180-550

Interpretive Information

- Dexamethasone administration

Clinical Background

Dexamethasone is a potent synthetic glucocorticoid widely used in medicine. The dexamethasone suppression test (DST) is a standard diagnostic tool for Cushing's disease and has been applied to the evaluation of hypothalamic-pituitary-adrenal axis function in patients with psychiatric disease. The measurement of dexamethasone in serum allows the physician to assess compliance during therapy or when evaluating DST results.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 20 ng/dL

Specimen Requirements

0.85 mL refrigerated serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable

Collect specimen between 8 and 10 AM.

DHEA (Dehydroepiandrosterone)

410X
38954X

Clinical Use

- Marker of adrenal androgen production
- Assess adrenal reserve after ACTH stimulation

Reference Range

Serum (410X)		ng/dL
Men		180-1250
Women		130-980
Pregnancy		135-810
Post-ACTH stimulation		60 min
Men and women		545-1845
Children*		
Premature infants (31-35 wk)		≤3343
Term infants (1st wk of life)		≤761
ACTH stimulation	Baseline ng/dL	60 min ng/dL
<1 y	26-585	18-1455
1-5 y	9-42	21-98
6-12 y	11-155	34-320
Tanner II-III		
Males	25-300	62-390
Females	69-605	95-885
Tanner IV-V		
Males	100-400	195-510
Females	165-690	325-1460

Urine (38954X)	µg/24-h	µg/g/creat
DHEA, Adults	21-2710	
DHEA, Men		24-1640
DHEA, Women		13-730
Creatinine		g/24-h
3-8 y		0.11-0.68
9-12 y		0.17-1.41
13-17 y		0.29-1.87
Adults		0.63-2.50

*Pediatric data from *J Clin Endocrinol Metab.* 1991;73:674-686.

Interpretive Information

↑
- Adrenal tumors
- Cushing's disease
- Congenital adrenal hyperplasia
- Premature adrenarche

↓
- Addison's disease
- Anorexia nervosa

Clinical Background

Dehydroepiandrosterone (DHEA) is a weak androgen synthesized by the adrenal cortex. It has a short half-life and is usually converted to dehydroepiandrosterone sulfate.

Excessive DHEA secretion can produce acne, hirsutism, and virilization via conversion to testosterone.

Method

Serum (410X)

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 3 ng/dL

Urine (38954X)

- Gas chromatography, mass spectrophotometry (GC/MS)

Specimen Requirements

Serum (410X)

1 mL refrigerated serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

Specify age and sex on test request form.

Urine (38954X)

5 mL frozen aliquot of a 24-h urine

2.1 mL minimum

Refrigerate during collection; do not use a preservative. Record 24-h volume on vial and request form.

DHEA Sulfate 402X

Clinical Use

- Marker for adrenal cortical function and disease

Reference Range

Men	µg/dL	µg/dL
18-29 y		110-510
30-39 y		110-370
40-49 y		45-345
50-59 y		25-240
60-69 y		25-95
70-90 y		≤75
Women		
18-29 y		45-320
30-39 y		40-325
40-49 y		25-220
50-59 y		15-170
60-69 y		≤185
70-90 y		≤90
Children	Male	Female
0-1 mo*	≤316	15-261
1-6 mo*	≤58	≤74
7-12 mo*	≤26	≤26
1-3 y*	≤15	≤22
4-6 y*	≤27	≤34
7-9 y	≤91	≤92
10-13 y	≤138	≤148
14-17 y	38-340	37-307
Tanner stages		
I	≤89	≤46
II	≤81	15-113
III	22-126	42-162
IV	33-177	42-241
V†	110-510	45-320

*From *Clin Chem.* 1993;39(6):1171 [abstract 0243].
†Same as adults aged 18-29 years.

Interpretive Information

↑
- Congenital adrenal hyperplasia
- Adrenal carcinoma
- Virilizing tumors of the adrenals
- Cushing's disease, pituitary dependent

↓
- Addison's disease
- Adrenal hypoplasia

Clinical Background

Dehydroepiandrosterone sulfate (DHEA-S), which is synthesized almost exclusively by the adrenals, is a weak androgen, the most abundant C19 plasma steroid, and the major source of urinary 17-ketosteroids. Measuring serum DHEA-S, therefore, can replace older urinary 17-ketosteroid determinations.

DHEA-S levels are markedly increased in patients with congenital adrenal hyperplasia or adrenal carcinoma. Moderate increases are seen in the majority of patients with pituitary-dependent Cushing's disease, whereas low or normal levels are usually exhibited by patients with Cushing's syndrome due to an adrenal adenoma. Adrenal cancer is typically associated with very high levels of DHEA-S. Since DHEA-S levels reflect adrenal androgen production, the measurement of serum concentrations serves as an early indicator for the onset of adrenarche.

Serum DHEA-S measurements are most commonly employed in the differential diagnosis of a virilized patient. In patients with virilizing tumors, DHEA-S levels usually exceed 7,000 µg/dL.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 15 µg/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Specify age and sex on test request form.

Dihydrotestosterone (DHT) 204X

Clinical Use

- Assess pseudohermaphroditism
- Diagnose 5α-reductase deficiency
- Assess efficacy of drugs that inhibit 5α-reductase

Reference Range

	ng/dL	ng/dL
Men		25-75
Women		5-30
Children	Males	Females
Cord blood	<2-8	<2-5
1-6 mo	12-85	<5
Prepubertal	<5	<5
Tanner II-III	3-33	5-19
Tanner IV-V	22-75	3-30

Includes data from *J Clin Endocrinol Metab.* 1979;48:821-826.

Interpretive Information

↑
- Hirsutism
- Chronic anovulatory syndrome
- High 5α-reductase activity

↓
- Hypogonadism
- 5α-reductase deficiency
- Drugs that reduce 5α-reductase activity (finasteride, etc)

Clinical Background

Dihydrotestosterone (DHT) is a potent androgen largely derived from the peripheral tissue conversion of testosterone, catalyzed by the enzyme 5α- reductase. Small amounts of DHT are secreted by the testes. DHT has potent androgenic properties. DHT also is produced in females from testosterone and androstenediol. DHT exerts its androgenic activity by binding to testosterone receptors in selective target tissues. It is an important bioactive androgen for male external genitalia development, prostate growth, and skin metabolism.

DHT is a causal agent in prostate hyperplasia, and measurements in blood can be used to assess compliance and response to inhibitors of testosterone-to-DHT conversion.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 3 ng/dL

Specimen Requirements

4 mL refrigerated serum

1.1 mL minimum

No additive red top preferred

SST red top acceptable

Specify age and sex on test request form.

Dihydrotestosterone, Free 36168X

Clinical Use

- Assess pseudohermaphroditism
- Diagnose 5α-reductase deficiency
- Assess efficacy of drugs that inhibit 5α-reductase

Reference Range

DHT, Total	ng/dL
Men	25-75
Women	5-30
DHT, Free	pg/mL
Men	1.0-6.2
Women	0.3-1.9
% DHT, Free	%
Men	0.62-1.10
Women	0.47-0.68

Interpretive Information

↑
- Hirsutism
- Chronic anovulation
- High 5α–reductase activity

↓
- Hypogonadism
- 5α-reductase deficiency
- Drugs inhibiting 5α–reductase

Clinical Background

Most dihydrotestosterone (DHT) is transported in the circulation bound to sex hormone binding globulin (SHBG). Approximately 1%, however, circulates unbound. Blood levels are dependent on rates of production, metabolic clearance, and binding protein concentration. Because SHBG levels are altered by medications, disease, and sex steroids, as well as insulin, measurement of free DHT more accurately reflects the level of bioactive DHT than measurements of total serum DHT.

Method

- Equilibrium dialysis, extraction, chromatography, radioimmunoassay (RIA)

Specimen Requirements

5 mL refrigerated serum (no additive red top tube); 2.5 mL minimum

Estradiol, Free 36169X

Clinical Use

- Assess estrogen status in males and females

Reference Range

Estradiol	pg/mL
Men	≤29
Women	
Follicular	39-375
Midcycle peak	94-762
Luteal	48-440
Postmenopausal	≤10
Estradiol, Free	
Men	≤0.45
Women	
Follicular	0.43-5.03
Midcycle peak	0.72-5.89
Luteal	0.40-5.55
Postmenopausal	≤0.38
Estradiol, Free	%
Men	1.25-1.85
Women	
Follicular	0.65-1.79
Midcycle peak	0.36-2.34
Luteal	0.56-1.87
Postmenopausal	0.79-1.64

Interpretive Information

- Estrogen secreting tumors
- Male hypogonadism (gynecomastia)
- Liver disease

- Ovarian failure
- Postmenopause

Clinical Background

More than 95% of circulating estrogens, including estradiol, are bound by serum binding proteins such as sex hormone binding protein (SHBG), corticosteroid binding protein (CGB), and albumin. The most important is SHBG, which is produced by the liver and binds steroid hormones with high affinity. Estrogens and thyroid hormones increase SHBG concentrations, while androgens reduce hepatic production. SHBG levels also are altered by disease and medications. CBG concentrations, also of hepatic origin, are influenced by hormones, disease, and medications. Dialyzable estradiol concentration most reliably reflects true hormone status.

Method

Estradiol

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 2 pg/mL

Estradiol, Free

- Equilibrium dialysis

Specimen Requirements

1.0 mL refrigerated serum (no additive red top tube); 0.5 mL minimum

SST red top unacceptable

Estradiol, Ultra Sensitive 30289X

Clinical Use

- Determine estrogen status in women
- Monitor follicular development during induction of ovulation
- Assess estrogen production in males

Reference Range

	pg/mL	pg/mL
Men		≤29
Women		
Follicular phase		39-375
Midcycle peak		94-762
Luteal phase		48-440
Postmenopausal		≤10

Children	Males	Females
Pre-pubertal (1-9 y)	≤4	≤16
10-11 y	≤12	≤65
12-14 y	≤24	≤142
15-17 y	≤31	≤283

Interpretive Information

Increased:
- Ovarian tumors
- Adrenal feminizing tumors
- Precocious puberty (female)
- Liver disease
- Male gynecomastia

Decreased:
- Ovarian failure
- Oral contraceptives

Clinical Background

Estradiol-17β (E2) is the major bioactive estrogen produced in the ovary. E2 is also produced by the adrenal glands, and in males by the testes, as well as by peripheral conversion from testosterone.

Serum E2 is measured to determine the estrogen status of women, such as in some cases of amenorrhea, and as a guide to monitoring follicular development during induction of ovulation.

The assay has high sensitivity and is therefore well suited for measurements in children and for diagnosing menopause.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 2 pg/mL

Specimen Requirements

0.5 mL refrigerated serum (no additive red top tube); 0.2 mL minimum

SST red top unacceptable

Specify age and sex on test request form.

Estrogen, Total, Serum 439X

Clinical Use

- Determine estrogen status in women
- Assess estrogen production in males
- Screen for estrogen-producing tumors

Reference Range

	pg/mL
Males	≤130
Females	
Early follicular	70-400
Late follicular	100-900
Luteal	70-700
Postmenopausal	≤130

This assay is not recommended for use in pre-pubertal children.

Interpretive Information

- Ovarian tumors
- Adrenal feminizing tumors
- Precocious puberty (female)
- Male gynecomastia

- Ovarian failure
- Oral contraceptives

Clinical Background

Estrogens are 18-carbon steroids secreted by the gonads, adrenal glands, and placenta. The principal estrogens are estradiol (E2), estrone (E1), and estriol (E3). E2 is the major estrogen produced by the ovaries. E1 is derived from ovarian secretion and peripheral conversion from gonadal and adrenal androstenedione. E3 is produced in the liver by metabolism from E2. The placenta produces all of these estrogens. This assay measures E1 and E2.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 25 pg/mL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Estrogens, Fractionated, Serum 36742X

Clinical Use

- Assess estrogen status

Reference Range

	pg/mL	pg/mL
Estrone (E1)		
Men		≤68
Women		
Follicular		10-138
Midcycle		49-268
Luteal		16-173
Postmenopausal		≤65
Children	Males	Females
Pre-pubertal (1-9 y)	<10	≤34
10-11 y	≤12	≤72
12-14 y	≤28	≤75
15-17 y	≤64	≤188
Estradiol (E2)		
Men		≤29
Women		
Follicular		39-375
Midcycle		94-762
Luteal		48-440
Postmenopausal		≤10
Children	Males	Females
Pre-pubertal (1-9 y)	≤4	≤16
10-11 y	≤12	≤65
12-14 y	≤24	≤142
15-17 y	≤31	≤283
Estriol (E3)		ng/mL
Men		≤0.2
Women		
Non-pregnant		≤0.1
Weeks gestation		
6-16.5		0.1-1.60
17.0-24.0		1.5-5.8
25.0-32.5		2.7-9.0
33.0-40.0		7.2-28.0

Interpretive Information

- Ovarian tumors
- Feminizing adrenal tumors
- Precocious puberty

- Ovarian failure
- Postmenopausal
- Gonadotropin deficiency

Clinical Background

Estrogens are 18-carbon steroids secreted by the gonads, adrenal glands, and placenta. The principle estrogens are estradiol (E2), estrone (E1), and estriol (E3). E2 is the major estrogen produced by the ovaries. E1 is derived from peripheral conversion from gonadal and adrenal androstenedione. E3 is the major placental estrogen. The major circulating estrogens in non-pregnant women are E2 and E1.

Method

Estrone, Estradiol

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)

Estriol

- Extraction, chromatography, radioimmunoassay (RIA)

Specimen Requirements

3 mL refrigerated serum (no additive red top tube); 0.8 mL minimum

SST red top unacceptable

Estrone, Serum 23244X

Clinical Use

- Assess estrogen status in post-menopausal women
- Assess placental estrogen production

Reference Range

	pg/mL	pg/mL
Men		≤68
Women		
Follicular		10-138
Midcycle		49-268
Luteal		16-173
Postmenopausal		≤65
Children	Males	Females
Pre-pubertal (1-9 y)	<10	≤34
10-11 y	≤12	≤72
12-14 y	≤28	≤75
15-17 y	≤64	≤188

Interpretive Information

- ↑ Pregnancy
- ↑ Postmenopause (by peripheral conversion)
- ↑ Aging and obesity
- ↑ Luteal phase of menstrual cycle
- ↓ Hypogonadism

Clinical Background

Estrone (E1) is 1 of 2 major ovarian estrogens in nonpregnant women and a major estrogen produced by the placenta. The primary ovarian estrogen is estradiol (E2). Circulating E1 is derived from ovarian secretion and peripheral conversion from gonadal and adrenal androstenedione. E1 has lower estrogenic potency than E2. E1 is conjugated to glucuronide or sulfate by the liver and excreted in urine and bile. The extragonadal conversion of androstenedione to E1, catalyzed by P-450 aromatase, occurs largely in lipid tissue; thus, E1 tends to be increased in obese subjects. Extragonadal conversion is the major source of circulating estrogens in postmenopausal women. A change in the estradiol/estrone ratio is a reflection of ovarian senescence and development of menopause.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 10 pg/mL

Specimen Requirements

0.5 mL refrigerated serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable

Specify age and sex on test request form.

Estrone Sulfate 37104X

Clinical Use

- Monitor estrogen replacement therapy

Reference Range

	pg/mL
Men	230-2200
Women	
Follicular phase	300-2600
Luteal phase	100-3200
Postmenopausal	100-1300

Interpretive Information

↑
- Pregnancy
- Ovarian tumors
- Adrenal tumors

↓
- Hypogonadism

Clinical Background

Estrone sulfate (E_1S) is the most abundant estrogen in peripheral blood. The primary source is the liver, where it is formed as the primary product of estradiol and estrone metabolism. E_1S also is the major estrogen secreted by the adrenal cortex. It is a weak estrogen but can be converted in the body to estradiol. E_1S is a major component of the drug Premarin®.

Method

- Hydrolysis, extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 40 pg/mL

Specimen Requirements

2 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Fructosamine 8340X

Clinical Use

- Monitor glucose control (short-term, 1-2 weeks) in patients with diabetes
- Manage patients with gestational diabetes

Reference Range

Adults	190-270 µmol/L
Children	
>5 y	Similar to adults

Pediatric data from *Ann Clin Biochem.* 1989;26:328-331; *Arch Dis Child.* 1986; 61:113-117; and *Clin Chem.* 1988;34: 2444-2447.

Interpretive Information

- Uncontrolled diabetes
- Hyperglycemia

- Relatively, with improved diabetic control

Clinical Background

Glucose forms stable glycoproteins with many plasma proteins by a nonenzymatic reaction. The glycosylated serum proteins, primarily albumin, have a short half-life of 1 to 2 weeks. Fructosamine determination is based on measurement of glycosylated serum proteins and is therefore useful for monitoring short-term glycemic control (eg, determining the success of a change in therapy in less time than is possible with glycated hemoglobin). It can also be used separately or in conjunction with blood glucose and/or glycosylated hemoglobin in diabetes management, especially in pregnancy-associated diabetes in which there is a need for careful monitoring of blood sugar control.

Since abnormal hemoglobins do not interfere with the fructosamine determination, it can also be used to monitor patients with abnormal hemoglobin, (eg, sickle cell anemia) and gestational diabetes.

Method

- Colorimetry
- Analytical sensitivity: 5.0 µmol/L

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

FSH (Follicle Stimulating Hormone) 470X

Clinical Use

- Diagnose gonadal function disorders

Reference Range

	IU/L
Men	1.6-8.0
Women	
Follicular phase	2.5-10.2
Midcycle	3.1-17.7
Luteal phase	1.5-9.1
Postmenopausal	23.0-116.3
Pediatric	
Use test code 36087X	

Interpretive Information

↑
- Primary hypogonadism
- Gonadotropin-secreting pituitary tumors
- Menopause

↓
- Hypothalamic GnRH deficiency
- Pituitary FSH deficiency
- Ectopic steroid hormone production

Clinical Background

Follicle stimulating hormone (FSH, follitropin) is a glycoprotein produced by the anterior pituitary gland. Production is regulated by hypothalamic gonadotropin releasing hormone (GnRH) and feedback from gonadal steroid hormones. In the female, FSH stimulates follicular growth, prepares ovarian follicles for luteinizing hormone (LH) activity, and enhances the LH-induced release of estrogen. After menopause, decreased ovarian estradiol secretion results in increased FSH and LH levels. In the male, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Primary testicular failure results in increased FSH and LH levels.

In patients with testicular or ovarian disorders, low levels of serum FSH are indicative of pituitary or hypothalamic dysfunction. Assays for both FSH and LH are useful in the diagnosis, management, and treatment of infertility in both genders.

Method

- Immunochemiluminometric assay (ICMA), Centaur
- Analytical sensitivity: 0.07 IU/L

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

FSH (Follicle Stimulating Hormone), Pediatrics 36087X

Clinical Use

- Diagnose and manage pubertal disorders
- Diagnose (including differential diagnosis) gonadal dysfunction
- Monitor FSH suppressive therapy

Reference Range

Males	IU/L
0-9 y	<3.0
10-13 y	0.3-4.0
14-17 y	0.4-7.4
Females	
0-8 y	0.5-4.5
9-13 y	0.4-6.5
14-17 y	0.8-8.5

In male infants, FSH peaks (typically 3.0-6.0 IU/L using this assay) at 4 months of age, falling to prepubertal levels by 1 year of age. In female infants, FSH peaks (as high as 30.0 IU/L using this assay) at 3 months of age, falling slowly to prepubertal levels by 1-2 years of age (Forest MG, Ducharme JR. Gonadotropic and gonadal hormones. In: Bertrand J, Rappaport R, Sizonenko PC, eds. *Pediatric Endocrinology*. 2nd ed. Baltimore, MD: Williams & Wilkins; 1993:100-120).

Interpretive Information

↑
- Precocious puberty
- Primary hypogonadism
- Gonadotropin-secreting pituitary tumors
- Menopause
- Germinal cell aplasia

↓
- Delayed puberty
- Hypothalamic GnRH deficiency
- Pituitary insufficiency
- Isolated FSH deficiency
- Isolated gonadotropin deficiency
- Hyperprolactinemia

Clinical Background

Follicle stimulating hormone (FSH, follitropin) is produced by the anterior pituitary gland after stimulation by hypothalamic gonadotropin releasing hormone (GnRH). FSH stimulates testicular inhibin and sperm production in males and ovarian inhibin and estrogen production in females. GnRH and FSH production are regulated by negative feedback systems, whereby low levels of gonadal hormones stimulate and high levels inhibit circulating FSH levels.

FSH is secreted transiently during infancy, after which the GnRH pulse generator controlling secretion becomes quiescent. Secretion is reactivated during pubescence, the first sign of which is increased FSH and LH secretion at night. Puberty progresses as gonadotropin secretion increases, and increasing gonadal responsiveness is associated with sustained increases in circulating sex hormone levels.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.05 IU/L

Specimen Requirements

0.5 mL refrigerated serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Gastric Parietal Cell Antibody 15114X

Clinical Use

- Differential diagnosis of anemia
- Assess atrophic gastritis
- Evaluate polyglandular autoimmune syndromes

Reference Range

	Units
Negative	≤20
Equivocal	20.1-24.9
Positive	≥25

Interpretive Information

- Atrophic gastritis
- Pernicious anemia
- Polyglandular autoimmune disease

Clinical Background

Gastric parietal cell antibodies are directed primarily against the gastric proton pump, H/K ATPase. The antibody is detected in approximately 90% of patients with pernicious anemia and the underlying type A chronic atrophic gastritis, in 20% to 30% of patients with varied autoimmune endocrine disorders, and in 2.5% to 10% (varies with age) of the healthy population.

Approximately 50% of patients with pernicious anemia have thyroid autoantibodies, and 30% of patients with autoimmune thyroid disease have PCA.

Method

- Enzyme linked immunosorbent assay (ELISA)
- Analytical sensitivity: 20 Units

Specimen Requirements

0.5 mL refrigerated serum

0.1 mL minimum

No additive red top preferred

SST red top acceptable

Gastrin 478X

Clinical Use

- Diagnose gastrinoma
- Differential diagnosis of peptic ulcer disease and gastrin-secreting tumors

Reference Range

	pg/mL
Adults	≤100
Children	
5-7 y	13-64

Interpretive Information

- Gastrinoma
- Pernicious anemia
- Atrophic gastritis with anacidity

Zollinger-Ellison syndrome: gastrin levels >200 pg/mL (Roy PK, et al. *Medicine*. 2000;79:379-411.)

Zantac®, Prilosec®, and pepsid interfere with gastrin production.

Clinical Background

Gastrin is a polypeptide hormone secreted by the G cells of the gastric antral mucosa and proximal duodenal cells. Gastrin is a potent stimulator of antral acid secretion, and also has trophic actions on the parietal cells, chief cells, and enterochromaffin-like cells in the oxyntic mucosa. Gastrin levels increase with food intake (protein, amino acids) and age. Levels decrease with gastric acid secretion.

In Zollinger-Ellison syndrome (ZE syndrome), a pancreatic neoplasm produces large amounts of gastrin, which in turn stimulates excess gastric acid secretion and peptic ulcer disease. A variety of pathological, but non-tumorous, disorders in which gastric acid secretion is low also lead to elevations in blood gastrin; these disorders include pernicious anemia and atrophic gastritis with anacidity. Secretin- or calcium-stimulation tests can assist in differentiating tumors from gastrin secreted by the antrum in patients with slight or moderate hypergastrinemia.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 10 pg/mL
- Analytical specificity: detects gastrin (G17); 7% cross reactivity with big gastrin (G34); does not cross react with G13.

Specimen Requirements

1 mL frozen serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is required.

Glucagon 519X

Clinical Use

- Diagnose glucagonoma
- Diagnose glucagon deficiency in patients with hypoglycemia

Reference Range

	pg/mL
Adults	≤60
Children	
Cord blood	≤215
Day 1	≤240
Day 2	≤400
Day 3	≤420
Days 4-14	≤148

Pediatric data from *J of Clin Invest.* 1974; 53:1159-1166 and *Pediatr Res.* 1981;15: 912-915.

Interpretive Information

- Newborn
- Glucagonoma
- Diabetes mellitus (relative or actual)

- Diabetes mellitus (occasional)
- Hypoglycemia (rare)

Clinical Background

Glucagon is produced by alpha cells of the pancreatic islets. Like insulin, glucagon secretion is regulated by blood glucose concentrations; however, regulation is reciprocal in that high glucose levels stimulate insulin and inhibit glucagon release. Conversely, low insulin levels stimulate glucagon secretion. Glucagon secretion also is stimulated by a high-protein meal.

Glucagon acts to increase blood sugar by stimulating hepatic glycogenolysis and gluconeogenesis, augmenting ketogenesis, and inhibiting lipogenesis. Thus, glucagon and insulin provide a bihormonal regulation of blood glucose concentration.

The plasma glucagon assay is useful primarily when considering a glucagon-secreting tumor of the pancreas. Glucagonomas cause an unusual but characteristic syndrome consisting of a rash (migratory necrolytic dermatitis located in the groin, around the mouth and nose, and in intertriginous areas), mild diabetes, weight loss, and hypoaminoacidemia. Measurement of plasma glucagon confirms the diagnosis; glucagon levels are very high (>1,000 pg/mL) in the setting of a glucagonoma.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 50 pg/mL

Specimen Requirements

3 mL room temperature EDTA plasma

1.1 mL minimum

Overnight fasting is required.

Glucose, Serum 483X

Clinical Use

- Assess carbohydrate metabolic status
- Diagnose diabetes mellitus
- Diagnose hypoglycemia

Reference Range

Adolescents and adults*	mg/dL
Normal	65-99
Impaired	100-125
Diabetes	>125

*Fasting

Interpretive Information

- Diabetes mellitus
- Cushing's syndrome
- Pheochromocytoma

- Islet cell tumor
- Glucagon deficiency
- Addison's disease
- Hypoglycemic syndromes

Clinical Background

Blood glucose concentrations are narrowly maintained by balancing hormonal factors. Insulin promotes glucose utilization and storage, lowering blood levels. Carbohydrate ingestion raises blood glucose concentrations; peak levels are modulated by stimulated insulin secretion. Several counter regulatory hormones (glucagon, growth hormone, cortisol, catecholamines) function to increase glucose concentrations by stimulating glycogenolysis, gluconeogenesis and increased hepatic glucose output, raising blood glucose at the expense of carbohydrate and other substrate stores.

Method

- Spectrophotometry (hexokinase)
- Analytical sensitivity: 2 mg/dL

Specimen Requirements

1 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

Glutamic Acid Decarboxylase-65 Autoantibodies (GAD-65) 34878X

Clinical Use

- Assess risk of type 1 diabetes
- Predict onset of type 1 diabetes
- Diagnose new onset type 1 diabetes

Reference Range

≤1 U/mL

Interpretive Information

- Type 1 diabetic patients
- Patients at risk for developing type 1 diabetes
- Stiff-man syndrome (SMS)

Clinical Background

Type 1 diabetes mellitus is characterized by lymphocytic infiltration of islet cells and circulating autoantibodies against a variety of islet cell antigens (ICA). These include glutamic acid decarboxylase (GAD), insulin, IA-2, GM2-1, and others. Direct assays for autoantibodies to GAD-65, IA-2, and insulin are now available for detecting individuals with a genetic predisposition to type 1 diabetes.

GAD-65 autoantibodies are observed in 70% to 80% of prediabetic and type 1 diabetic subjects including 7% to 8% of adult onset diabetics with type 1 diabetes. When a positive GAD-65 antibody test is coupled with positive autoantibody tests for IA-2 and insulin, the risk of diabetes increases to >90% (see Test Application and Interpretation section).

Method

- Radiobinding assay
- Analytical sensitivity: 0.2 U/mL

Specimen Requirements

1 mL frozen serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Glycated Albumin 5032X

Clinical Use

- Assess glucose control (short-term, 1-2 weeks) in patients with diabetes, especially those with pregnancy-associated diabetes

Reference Range

Adults and children	0.8-1.4%

Interpretive Information

- Uncontrolled diabetes
- Hyperglycemia
- Microvascular disease/diabetic complications

- Relatively, with improved diabetic control

Clinical Background

Glucose forms stable glycoproteins with many plasma proteins by a nonenzymatic reaction. Among the plasma proteins, albumin is quantitatively the most important glycated protein. Concentrations of glycated albumin are high in diabetic patients and have been shown to be proportional to the degree of hyperglycemia. Since albumin turns over in 1 to 2 weeks, glycated albumin determinations are useful for monitoring short-term glucose control in diabetic patients. In contrast, glycated hemoglobin, which has a half-life of 120 days, is best for assessment of long-term control in such patients.

Method

- Affinity column chromatography
- % glycated albumin is based on percentage of microalbumin relative to amount of total albumin.

Specimen Requirements

3 mL refrigerated EDTA plasma

2 mL minimum

Overnight fasting required

Growth Hormone (GH) 521X

Clinical Use

- Assess pituitary growth hormone disorders

Reference Range

	ng/mL
Adults	
Fasting	≤10
After 75 g oral glucose	<1
Children	≤13

Interpretive Information

↑
- Gigantism
- Acromegaly
- Selected pituitary tumors
- Pregnancy, due to HPL cross-reactivity
- Laron dwarfism (GH resistance)

↓
- Pituitary GH deficiency
- Hypopituitarism, congenital or acquired
- GH secretory dysfunction

Because of diurnal variability, random growth hormone values are not reliable for diagnosis of growth hormone deficiency, acromegaly (adults), or gigantism (pediatrics).

Clinical Background

GH is a polypeptide (~ 20,000 MW) secreted by the anterior pituitary gland under the control of hypothalamic growth hormone releasing hormone (GHRH) and somatostatin, which stimulate and inhibit release, respectively.

Excess GH production can be caused by pituitary adenoma. In adults, excess GH production produces acromegaly; in children, it produces gigantism. Insufficient GH production in children results in growth retardation and, in adults, decreased muscle mass.

Measuring GH concentration in children is useful for clinically evaluating short stature and helping to differentiate between abnormal GH production and other causes of growth failure. In adults a GH deficiency syndrome has been described that is associated with osteoporosis, dyslipidemia, increased visceral obesity, and poor quality of life. Because random GH measurements are not usually meaningful, inhibitory protocols, provocative tests, and prolonged sampling usually are employed. See Disorders of Growth Hormone in the Test Application and Interpretation section.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.1 ng/mL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Growth Hormone (GH) Antibody 37072X

Clinical Use

- Monitor patients receiving GH therapy

Reference Range

Adults and children	Negative

Interpretive Information

- Selected patients on GH therapy
- Possibly in patients with autoimmune hypophysitis

Clinical Background

Human GH is now a routine replacement therapy in GH-deficient patients. Most of the GH used is biosynthetically produced with recombinant DNA technology and is purified to minimize antigenicity. Nonetheless, some patients will develop autoantibodies; these may not be neutralizing and may not inhibit the growth response. About 5% of patients will produce neutralizing antibodies; thus, all patients with documented GH deficiency who do not respond as expected to GH therapy should receive GH antibody testing.

Method

- Radiobinding assay (RBA)
- Titer reported when antibody is present

Specimen Requirements

0.5 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Growth Hormone-Binding Protein (GHBP) 39573X

Clinical Use

- Diagnose Laron dwarfism
- Differential diagnosis of growth hormone resistance
- Indicate GH responsiveness

Reference Range

	pmol/L
Adults	400-4260
Children	
3-8 y	320-3820
9-13 y	240-2890
14-17 y	290-3140

Interpretive Information

- Estrogen treatment
- Familial short stature with high GHBP (*J Clin Endocrinol Metab.* 2004;89:1031-1044.)

- Laron dwarfism

Clinical Background

Human growth hormone (GH) exists in several isometric forms in serum – either free or bound to a specific binding protein (GHBP). This protein is identical to the extracellular domain of the GH receptor and appears to be derived from the GH receptor gene by alternative splicing. Levels vary with age, being low in neonates and highest in young adults. Normal or elevated levels in patients with classical Laron syndrome indicate a defect in the transmembrane, intracellular, or post GH receptor domains. Very low or absent GHBP denotes a defect in the extracellular domain. A low serum GHBP in relatives of patients with Laron syndrome helps identify heterozygous carriers of patients with defects in the extracellular domain.

Method

- Enzyme-linked immunosorbent assay (ELISA)
- Analytical sensitivity: 10 pmol/L

Specimen Requirements

1 mL frozen serum (no additive red top tube); 0.1 mL minimum

SST red top unacceptable

Growth Hormone Releasing Hormone (GHRH) 37557X

Clinical Use

- Differential diagnosis of acromegaly

Reference Range

	pg/mL
Adults	≤49
Children (4-14 y)	6.8-19.0

Pediatric data from *Horm Metabol Res.* 1987;19:434-436.

Interpretive Information

- Ectopic GHRH production
- Pregnancy (maternal and fetal samples)

Clinical Background

Growth hormone releasing hormone (GHRH) is secreted by the hypothalamus and stimulates the pituitary to release growth hormone. GHRH deficiency is probably the most common cause of growth hormone deficiency in man.

Acromegaly results from hypersecretion of growth hormone and is due, in over 99% of cases, to a primary pituitary tumor. The remaining cases result from GHRH hypersecretion due to hypothalamic tumor or peripheral tumors (pancreatic islet tumors, bronchial or thymic carcinoid tumors, and rarely neuroendocrine tumors). In patients with acromegaly due to ectopic GHRH secretion, peripheral GHRH levels are elevated.

The differential diagnosis between a pituitary tumor and ectopic GHRH hypersecretion is achieved by measuring peripheral GHRH levels. Circulating GHRH is nonhypothalamic in origin.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 16 pg/mL

Specimen Requirements

4 mL frozen plasma

1.1 mL minimum

Collect in special PTH-Related Protein (PTH-RP) and Releasing Factors tube provided by Quest Diagnostics Nichols Institute. Centrifuge immediately in refrigerated centrifuge, separate plasma, and freeze.

hCG, Total with HAMA Treatment 19720X

Clinical Use

- Diagnose pregnancy
- Diagnose chorionic tumors and ectopic hCG-producing tumors

Reference Range

hCG, Total, HAMA	<5 mIU/mL
hCG, Total	<5 mIU/mL

Interpretive Information

- Pregnancy
- Trophoblastic tumors
- Gonadal germ cell tumors
- Midline trophoblastic tumors

- Ectopic pregnancy (relative to uterine pregnancy)

Clinical Background

Heterophilic antibodies, defined as antibodies against animal immunoglobulins, can be a source of interference in many immunoassays. The most frequently characterized interference comes from endogenous human anti-mouse antibodies (HAMA), which can react with the mouse monoclonal antibodies utilized in the assay. HAMA presence in the patient sample can lead to over- or underestimation of human chorionic gonadotropin (hCG). In this test, HAMA is removed by precipitation prior to hCG determination.

Method

- HAMA precipitation
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 2 mIU/mL
- Analytical specificity: detects intact and free beta hCG

Specimen Requirements

2 mL room temperature serum

1.0 mL minimum

No additive red top preferred

SST red top acceptable

hCG, Total, Quantitative 8396X

Clinical Use

- Diagnose pregnancy
- Diagnose chorionic tumors and ectopic hCG-producing tumors

Reference Range

	mIU/mL
Men	<5
Women	
Non-pregnant, premenopausal	<5
Postmenopausal	<10
Pregnant	Expected*
<1 wk	5-50
1-2 wk	50-500
2-3 wk	100-5,000
3-4 wk	500-10,000
4-5 wk	1,000-50,000
5-6 wk	10,000-100,000
6-8 wk	15,000-200,000
2-3 mo	10,000-100,000

*Bayer Centaur product insert

Values from different assay methods may vary.

Interpretive Information

↑
- Pregnancy
- Trophoblastic tumors
- Gonadal germ cell tumors
- Midline trophoblastic tumors

↓
- Ectopic pregnancy (relative to uterine pregnancy)

Clinical Background

Human chorionic gonadotropin (hCG) is secreted by the developing placenta often as early as 6 days after conception; maximal production and peak serum levels occur during the first trimester but remain significant throughout gestation. The immunochemiluminometric assay for serum hCG provides a fast and accurate test for pregnancy. Because the assay is highly sensitive, it is useful for early determination of pregnancy when hCG levels are relatively low.

A variety of other tissues are capable of hCG production, and increased levels in men or in nonpregnant women suggest neoplasia. In this context, hCG levels >10,000 mIU/mL occur only in germ cell tumors, in patients with trophoblastic differentiation of a lung or gastric primary cancer, or in women with gestational trophoblastic disease. Levels are prognostic in germ cell tumors: <5,000 mIU/mL indicates good prognosis; 5,000 to 50,000 mIU/mL indicates intermediate prognosis; and >50,000 mIU/mL indicates poor prognosis.

hCG measurement also is appropriate in the evaluation of men with gynecomastia and boys with isosexual precocious puberty to diagnose testicular malignancies.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 2 mIU/mL
- Analytical specificity: detects intact and free beta hCG

Specimen Requirements

1 mL refrigerated serum

0.4 mL minimum

No additive red top preferred

SST red top acceptable

Hemoglobin A_{1c} 496X

Clinical Use

- Monitor glucose control (long-term, 2-3 months) in patients with diabetes

Reference Range

Non-diabetic	<6.0%

Interpretive Information

Increased

- Diabetes mellitus
- Chronic hyperglycemia
- Presence of hemoglobin S (sickle cell)
- Presence of hemoglobin C variant

Decreased

- Relatively decreased by improved diabetic control
- High levels of hemoglobin F

Clinical Background

Hemoglobin A_1 (HbA_1 or glycated hemoglobin) is structurally related to adult hemoglobin (HbA) but has a glucose molecule attached to the terminal valine of the beta chain. Glycosylation is a nonenzymatic, irreversible process dependent on the glucose concentration and the duration of exposure of the erythrocyte to glucose. HbA_1 is continuously formed during the 120-day life of the erythrocyte, and a single measurement of HbA_1 reflects the average blood glucose level during the preceding months. HbA_1 can be separated into HbA_{1a}, HbA_{1b}, and HbA_{1c} fractions. HbA_{1c} correlates best with high glucose concentrations.

Method

- Colorimetry, immunoturbidimetric
- Analytical sensitivity: 3% at a hemoglobin concentration of 8.2 mmol/L (13.2 g/dL)

Specimen Requirements

2 mL refrigerated whole blood

0.3 mL minimum

Collect in EDTA (lavender-top) tube.

5-HIAA (5-Hydroxyindoleacetic Acid), Urine

39625X
1648X

Clinical Use

- Diagnose carcinoid tumors

Reference Range

5-HIAA (39625X)	mg/24-h
2-10 y	≤8
>10 y	≤6
Creatinine (39625X)	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50
5-HIAA (1648X)	mg/g creat
2-10 y	≤12
>10 y	≤10
Creatinine (1648X)	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Male	20-370
Female	20-320

Interpretive Information

↑ • Carcinoid tumor

Clinical Background

Serotonin metabolism yields 5-HIAA as a major urinary metabolite. Because carcinoid tumors secrete large amounts of serotonin and 5-HIAA, tests for 5-HIAA are useful in their diagnosis. However, many foods (see below) and drugs also contain large amounts of serotonin and should be avoided at least 48 hours before collecting urine. Drugs to avoid include phenothiazines, glyceryl guaiacolate, reserpine, MAO inhibitors, and imipramine.

Method

- High-performance liquid chromatography (HPLC), electrochemical detection
- Analytical sensitivity: 0.2 mg/L
- Creatinine concentration reported

Specimen Requirements

10 mL room temperature aliquot of 24-h urine ***(39625X)*** or random urine ***(1648X)***; 5 mL minimum

Collect 24-h urine with 25 mL of 6N HCl. Add 6N HCl to random urine to maintain pH <3. Record 24-h urine total volume on vial and request form.

Patient preparation: avoid tobacco, tea, and coffee for 3 days prior; avoid food high in indoles, eg, avocado, banana, tomato, plum, walnut, pineapple, and eggplant.

Homovanillic Acid (HVA), Urine

39527X
6346X

Clinical Use

- Diagnose catecholamine-producing tumors

Reference Range

24-H urine (39527X)

Homovanillic acid	mg/24-h
3-8 y	0.5-6.7
9-12 y	1.1-6.8
13-17 y	1.4-7.2
Adults	1.6-7.5
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Random urine (6346X)

Homovanillic acid	mg/g creat
Birth to 6 mo	9.1-36
7-11 mo	11.2-33
1-2 y	8.5-38
3-8 y	2.1-23
9-12 y	1.1-12
Adults	1.4-5.3
Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Male	20-370
Female	20-320

Interpretive Information

- Neuroblastoma
- Ganglioblastoma

Clinical Background

Measurements of urinary vanillylmandelic acid (VMA) and homovanillic acid (HVA) are valuable in identifying patients with neuroblastoma. About 80% of patients with neuroblastoma will have elevated urinary VMA. Other metabolites of dopamine, such as urinary dopamine, HVA, and 3-methoxy-4-hydroxyphenylglycol (MHPG), are also usually elevated.

Neuroblastomas are malignant tumors occurring in children. About half of the cases occur before 2 years of age. Neuroblastomas secrete mostly precursors of norepinephrine (dopamine and its metabolites) and seldom produce hypertension. Patients usually present with an abdominal mass. Surgery and radiation therapies offer significant hope for survival. Early diagnosis is therefore essential.

Method

- High-performance liquid chromatography (HPLC), electrochemical detection
- Analytical sensitivity: 0.5 mg/L
- Creatinine concentration also reported

Specimen Requirements

10 mL room temperature aliquot of a 24-h urine ***(39527X)*** or random urine ***(6346X)***; 5 mL minimum

Collect 24-h urine in 25 mL 6N HCl; add 6N HCl to random urine to maintain pH <3. Record 24-h total volume on vial and request form.

Patient preparation: avoid alcohol, coffee, tea, tobacco, and strenuous exercise prior to collection; preferably abstain from medications for 3 days, but common antihypertensives (diuretics, ACE inhibitors, calcium channel blockers, alpha and beta blockers) cause minimal or no interference.

17-Hydroxycorticosteroids, 24-Hour Urine

15202X

Clinical Use

- Assess adrenocortical function
- Reflect cortisol secretion

Reference Range

17-Hydroxycorticosteroid	mg/24-h
Men	3.0-10.0
Women	2.0-6.0
Children	
1-2 y	0.5-2.5
3-4 y	1.0-4.0
5-6 y	1.0-4.8
7-8 y	1.0-5.6
9-10 y	1.0-7.0
11-12 y	1.5-8.0
13-16 y	
Males	2.0-6.0
Females	2.8-6.8
17-20 y	
Males	3.0-10.0
Females	2.0-7.0
Creatinine	**g/24-h**
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Cushing's syndrome
- Adrenal tumors
- Marked stress
  - Infection
  - Surgery
  - Burns, etc

↓
- Addison's disease
- ACTH deficiency
- Starvation
- Liver disease
- Renal failure

Prescription and over-the-counter medications may affect results.

Clinical Background

Urine 17-hydroxycorticosteroids (17-OHCS) include 17-hydroxycorticosterone derived from compound F (hydrocortisone) and 17-hydroxy-11-dehydrocorticosterone (derived from compound E, cortisone). The parent hormones regulate protein and carbohydrate metabolism and are involved in feedback control of ACTH secretion. Urine 17-OHCS levels reflect adrenal cortical activity.

Method

- Modified Porter-Silber reaction
- Analytical sensitivity: 0.1 mg/L
- Creatinine concentration also reported

Specimen Requirements

20 mL refrigerated aliquot of a 24-h urine; 10 mL minimum

Collect with 10 g of boric acid (preferred) or 25 mL 6N HCl or 50% acetic acid. Record total volume on vial and request form.

18-Hydroxycorticosterone 6595X

Clinical Use

- Differential diagnosis of primary hyperaldosteronism due to adrenal adenoma

Reference Range

Adults (8-10 AM)	ng/dL	ng/dL
Upright		5-80
Supine		4-37
Children		
Premature infants (31-35 wk)		≤380
Term infants (3 d)		≤942
ACTH stimulation	Baseline	60 min
1-12 mo	5-310	67-470
1-5 y	7-155	49-370
6-12 y	10-74	79-360
Tanner II-III		
Males	5-73	91-1475
Females	11-82	69-195
Tanner IV-V		
Males	14-62	73-205
Females	11-68	82-320

Pediatric data from *J Clin Endocrinol Metab.* 1992;75:1491-1496 and *J Clin Endocrinol.* 1989;69:1133-1136.

Interpretive Information

↑
- Adrenal adenoma

↓
- Adrenal P-450$_{c11}$ase deficiency
- Addison's disease

Clinical Background

Primary aldosteronism should be suspected whenever a patient has the triad of hypertension, hypokalemia, and inappropriate renal potassium wasting. The biggest challenge is the differential diagnosis between the more common adrenal adenoma and bilateral hyperplasia. Because adrenal adenomas increase the circulating levels of 18-hydroxycorticosterone (18-OH B), serum levels of 18-OH B are useful to differentiate them from bilateral adrenal hyperplasia. The cutoff point appears to be about 50 ng/dL of serum. Most values are around 100 ng/dL. The higher the level of this precursor, the greater the possibility that an adrenal adenoma is present.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 7 ng/dL

Specimen Requirements

3 mL refrigerated serum

1.1 mL minimum

No additive red top preferred

SST red top acceptable

6β-Hydroxycortisol, 24-Hour Urine 38071X

Clinical Use

- Marker for hepatic microsmal enzyme induction

Reference Range

6β–Hydroxycortisol	µg/24-h
Adults	18-460
Children*	
0-2 mo	3-6
2 mo-3 y	3-49
3-8 y	15-95
9-12 y	24-197
13-17 y	44-350
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

*Pediatric ranges from Lashansky G, Saenger P, Fishman K, et al. *J Clin Endocrinol Metab.* 1991;73:674-686.

Interpretive Information

- Hepatic drug toxicity

Clinical Background

6β-Hydroxycortisol, a metabolite of adrenal cortisol, reflects hepatic P-450 enzyme activity and has been useful as a marker of hepatic drug toxicity. It also is useful for assessing adrenal cortical function in the newborn. 6β-Hydroxycortisol is the major urinary cortisol metabolite in the neonatal period.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 µg/L
- Creatinine concentration also reported

Specimen Requirements

20 mL frozen aliquot of a 24-h urine

2 mL minimum

Collect urine with 10 g of boric acid or keep refrigerated during collection. Record total volume on vial and request form.

18-Hydroxycortisol, Free, 24-Hour Urine

11178X

Clinical Use

- Diagnose glucocorticoid remediable aldosteronism (GRA)

Reference Range

18-Hydroxycortisol	µg/24-h
Men	51-515
Women	43-295
Children	
1-4.9 y	6-193
5-9.9 y	16-240
10-13.9 y	19-480
14-17.9 y	17-500
Creatinine	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

- ↑ Glucocorticoid remediable aldosteronism
- ↓ Adrenal insufficiency

Clinical Background

GRA is a rare autosomal dominant disease usually characterized by hypokalemia and early onset of severe hypertension refractory to conventional antihypertensive therapies. It is caused by a chimeric duplication involving the 11β-hydroxylase *(CYP11B1)* and aldosterone synthase *(CYP11B2)* genes such that aldosterone secretion is solely regulated by ACTH. Urinary levels of 18-hydroxycortisol and 18-oxocortisol levels are uniquely elevated in GRA. Dexamethasone suppression and genetic testing also are useful for diagnosis. Some affected individuals have mild hypertension without hypokalemia but a suppressed plasma renin.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 µg/L

Specimen Requirements

10 mL refrigerated aliquot of a 24-h urine collection; 2.1 mL minimum

Collect urine in 10 g boric acid or refrigerate during collection.

21-Hydroxylase Antibody 37916X

Clinical Use

- Confirm autoimmune Addison's disease
- Identify adrenal gland involvement in PAS

Reference Range

Adults and children	<1.0 U/mL

Interpretive Information

- PAS, type I
- PAS, type II
- Isolated autoimmune Addison's disease

Clinical Background

Autoimmune Addison's disease is characterized by the presence of autoantibodies to adrenal cortical cell antigens. Recent studies have identified the adrenal biosynthetic enzyme P-450$_{c21}$ as one of these antigens. Autoimmune Addison's disease is part of 2 polyglandular autoimmune syndromes (PAS), type I and type II. Type II, including autoimmune thyroiditis and diabetes, is the more frequent.

This direct autoantibody assay for anti-21-hydroxylase is more sensitive and quantifiable than the anti-adrenal immunofluorescence (IFA) method. Measurement of other glandular autoantibodies provides additional diagnostic and management information.

Method

- Radiobinding assay (RBA)
- Analytical sensitivity: 1.0 U/mL

Specimen Requirements

1 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

17-Hydroxypregnenolone 8352X

Clinical Use

- Marker for adrenal 3β-hydroxysteroid dehydrogenase enzyme deficiency

Reference Range

	ng/dL	ng/dL
Adults		20-450
Post ACTH stimulation, 60 min, men and premenopausal women (follicular phase)		290-910
Children		
Premature infants (31-35 wk)		≤2409
Term infants (3 d)		≤830
ACTH stimulation	Baseline	60 min
1-12 mo	14-830	395-3290
1-5 y	10-100	45-740
6-12 y	11-190	70-660
Tanner II-III		
Males	20-360	88-675
Females	58-450	250-800
Tanner IV-V		
Males	32-300	220-860
Females	53-540	500-1600

Pediatric data from *J Clin Endocrinol Metab.* 1991;73:674-686 and *J Clin Endocrinol Metab.* 1989;69:1133-1136.

Interpretive Information

↑
- CAH due to 3β-hydroxysteroid dehydrogenase enzyme deficiency
- Hirsutism (polycystic ovary or idiopathic)
- Adrenal tumors

↓
- Adrenal insufficiency

Clinical Background

17-Hydroxypregnenolone, a Δ^5 steroid, is an intermediate in the steroid hormone biosynthetic pathways. It is formed by the hydroxylation of pregnenolone and metabolized to 17-hydroxyprogesterone or dehydroepiandrosterone (DHEA). 17-hydroxypregnenolone production is stimulated by ACTH and suppressed by dexamethasone.

Production is increased in patients with congenital adrenal hyperplasia (CAH) associated with deficiency of the 3β-hydroxysteroid dehydrogenase enzyme.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 10 ng/dL

Specimen Requirements

2 mL refrigerated serum

0.4 mL minimum

No additive red top preferred

SST red top acceptable

17-Hydroxyprogesterone, LC/MS/MS

17180X
17654X

Clinical Use

- Marker for adrenal $P450_{c21}$ (21-hydroxylase) enzyme deficiency

Reference Range

Adults (17180X)	ng/dL	ng/dL
Men		
18-30 y		32-307
31-40 y		42-196
41-50 y		33-195
51-60 y		37-129
Women		
Follicular phase		≤185
Midcycle phase		≤225
Luteal phase		≤285
Postmenopausal		≤45
Neonatal/Infant (17654X)*		
Premature infants (31-35 wk)		≤360
Term infants (3 d)		≤420
1-12 mo		11-170
1-4 y		4-115
5–9 y		≤90
10-13 y		≤169
14-17 y		16-283
ACTH stimulation	Baseline	60 min
1-12 mo	11-170	85-465
1-5 y	4-115	50-350
6-12 y	7-69	75-220
Tanner II-III		
Males	12-130	69-310
Females	18-220	80-420
Tanner IV-V		
Males	51-190	105-230
Females	36-200	80-225

*Includes data from *J Clin Metab.* 1991; 73:674-686; *J Clin Endocrinol Metab.* 1989; 69:1133-1136; and *J Clin Endocrinol Metab.* 1994;78:266-270.

Clinical Background

17-Hydroxyprogesterone (17-OHP) is an adrenal steroid intermediate in the biosynthesis of cortisol. Its synthesis from progesterone is catalyzed by the enzyme $P450_{c17;}$ it is then further metabolized to cortisol or androstenedione.

17-OHP measurement is appropriate when complete or partial 21-hydroxylase deficiency is suspected in 1) infants with features of adrenal insufficiency (hypotension, vomiting, fever, hypoglycemia, and hyperkalemia) or ambiguous genitalia and 2) women with clinical evidence of possible androgen excess, particularly Ashkenazi Jews who have a high prevalence of nonclassical 21-hydroxylase deficiency. Some authorities consider measurement of ACTH-stimulated 17-OHP levels essential to exclude the diagnosis. Cushing's disease may also cause elevated 17-OHP levels and should be excluded before glucocorticoid treatment of presumed nonclassical 21-hydroxylase deficiency is considered.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 8 ng/dL

Specimen Requirements

0.5 mL frozen serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable

Interpretive Information

↑
- Congenital adrenal hyperplasia (CAH), 21-hydroxylase deficiency
- Male pseudohermaphroditism ($P450_{c17}$ deficiency)
- Late-onset CAH
- Cushing's disease

↓
- Steroid treatment (cortisone, hydrocortisone)
- Addison's disease

Hydroxyproline, Free, Plasma 37558X

Clinical Use

- Assess bone matrix turnover

Reference Range

	µmol/L
0-30 d	0-91
31 d-24 mo	0-63
2-18 y	3-45
>18 y	0-53

Interpretive Information

↑
- Paget's disease
- Bone metastatic cancer
- Hyperparathyroidism
- Osteoporosis
- Glucocorticoid excess

↓
- Fluoride excess
- Bisphosphonate therapy
- Anabolic steroid therapy

Clinical Background

Collagen metabolism releases a variety of collagen constituents into the circulation. These include amino- and carboxy-terminal extension peptides, pyridinoline, and deoxypyridinoline cross-links, and hydroxyproline. Hydroxyproline in plasma appears as both peptide bound and free forms. Bone resorption is the major contributor to collagen turnover, and the constituent molecules, including hydroxyproline, are useful as markers of the rate of bone matrix resorption. Hydroxyproline is most useful in conditions associated with markedly increased bone resorption (Paget's disease, malignancy, hyperparathyroidism).

Method

- High performance liquid chromatography (HPLC)
- Analytical sensitivity: 1 µmol/L

Specimen Requirements

2.0 mL frozen sodium heparin plasma

0.5 mL minimum

Separate plasma within 30 minutes of collection and freeze.

Provide patient age, sex, clinical history, tentative diagnosis, and therapy during the last 3 days (eg, drugs, X-ray, infant formula, diet).

Hydroxyproline, Free and Total, 24-Hour Urine

17190X, 685X, 535X

Clinical Use

- Index for the rate of bone formation and turnover
- Assess efficacy of therapy for bone disease

Reference Range

Hydroxyproline, Free (685X, 17190X)	mg/24-h
Adults	≤2.7
Hydroxyproline, Total (535X, 17190X)	
Men	9-73
Women	7-49
Children*	
Premature infants, 3 wk old	6-35
Full-term infants	
3 d	8-20
1 mo	32-63
1-10 y	15-150
11-14 y	68-169
Creatinine (685X, 535X, 17190X)	g/24-h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

*Pediatric data from *J Pediatr Gastroent Nutr.* 1991;13:176-181; *J Clin Invest.* 1970; 49:716-729; *Pediatr Res.* 1967;1:266-270; *J Pediatr.* 1966;69:266-273; and *J Clin Invest.* 1962;41:1928-1935.

Interpretive Information

- Paget's disease
- Hyperparathyroidism (primary and secondary)
- Burns
- Psoriasis
- Acromegaly
- Inborn errors of metabolism
- Hydroxyprolinemia
- Familial aminoglycinuria

Clinical Background

Hydroxyproline is an amino acid present in appreciable quantities in collagen and excreted in the urine after collagen breakdown. Because urinary hydroxyproline is derived almost entirely from collagen, it reflects the rate of collagen catabolism. Small amounts of urinary hydroxyproline are present as free amino acids, while most is associated with small and large nondialyzable peptides. This combination is collectively referred to as total hydroxyproline.

Urine hydroxyproline levels are greatly increased in Paget's disease and to a lesser extent in hyperparathyroidism (primary and secondary). Elevated levels are also observed in patients with burns, psoriasis, and acromegaly. Urinary hydroxyproline has been suggested as a tumor marker for lymphomas, mammary carcinoma, and bone metastatic prostate carcinoma.

Levels of free hydroxyproline are increased in patients with inborn errors of metabolism, hydroxyprolinemia, and familial aminoglycinuria.

Method

- Colorimetry
- Analytical sensitivity: 0.5 µg/mL and 2 µg/mL, free and total, respectively
- Creatinine concentration also reported

Specimen Requirements

25 mL refrigerated aliquot of a 24-h urine; 2.1 mL minimum for free (***685X***); 2.1 mL minimum for total (***535X***); 4.2 mL minimum for free and total (***17190X***)

Collect with 25 mL of 6N HCl. Record total volume on vial and request form.

Avoid meat and collagen-derived therapeutics for 24 to 48 hours prior to collection.

Hydroxyproline, Free and Total, Random Urine

11258X
37407X

Clinical Use

- Index for the rate of bone formation and turnover
- Assess efficacy of therapy for bone disease

Reference Range

Hydroxyproline, Free (11258X)	µg/mL
Adults	≤1.6
Hydroxyproline, Total (37407X)	
Adults	≤60
Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Male	20-370
Female	20-320

Interpretive Information

- Paget's disease
- Hyperparathyroidism (primary and secondary)
- Burns
- Psoriasis
- Acromegaly
- Inborn errors of metabolism
- Hydroxyprolinemia
- Familial aminoglycinuria

Clinical Background

Hydroxyproline is an amino acid present in appreciable quantities in collagen and excreted in the urine after collagen breakdown. Because urinary hydroxyproline is derived almost entirely from collagen, it reflects the rate of collagen catabolism. Small amounts of urinary hydroxyproline are present as free amino acids, while most is associated with small and large nondialyzable peptides. This combination is collectively referred to as total hydroxyproline.

Urine hydroxyproline levels are greatly increased in Paget's disease and to a lesser extent in hyperparathyroidism (primary and secondary). Elevated levels are also observed in patients with burns, psoriasis, and acromegaly. Urinary hydroxyproline has been suggested as a tumor marker for lymphomas, mammary carcinoma, and bone metastatic prostate carcinoma.

Levels of free hydroxyproline are increased in patients with inborn errors of metabolism, hydroxyprolinemia, and familial aminoglycinuria.

Method

- Colorimetry
- Analytical sensitivity: 0.5 µg/mL and 2 µg/mL, free and total, respectively
- Creatinine concentration also reported

Specimen Requirements

25 mL refrigerated aliquot of a random or 2nd void urine; 2.1 mL minimum

Patient should be on a diet sufficiently low in collagen for 24 hours prior.

Hypertension, Endocrine 15026X

Clinical Use

- Detect treatable causes of low-renin hypertension in children and adults

Reference Range

	Males µg/24-h	Females µg/24-h
Cortisol	7-185	13-115
THF	280-2820	210-1680
5α-THF	100-2660	100-1250
18-Oxo-THF	≤42	≤10
α-Cortol	30-490	25-215
β-Cortol	8-340	17-215
Cortisone	19-245	24-160
THE	445-5960	330-3430
α-Cortolone	47-1400	90-1100
β-Cortolone	8-560	10-220
Tetrahydroaldosterone	6-79	6-79

THF, tetrahydrocortisol; THE, tetrahydrocortisone.

Interpretive Information

	µg/24-h*
Primary aldosteronism	
Tetrahydroaldosterone	>80
18-Oxo-THF	<10
Glucocorticoid-suppressible aldosteronism	
Tetrahydroaldosterone	>50
18-Oxo-THF	>10
Apparent mineralocorticoid excess (AME)	
Tetrahydroaldosterone	<10
Total cortisol metabolites†	<3000
(THF + 5α-THF)/THE	>3
Cortisol/cortisone	>1
Hypoaldosteronism	
Tetrahydroaldosterone	<10

*For all except THF+5α-THF/THE and cortisol/cortisone ratios.

†Total cortisone metabolites include THF, 5α-THF, THE, α- and β-cortolone, α- and β-cortol, cortisol, and cortisone.

Clinical Background

Primary aldosteronism manifests with hypertension, increased aldosterone levels, potassium loss, and suppression of the renin-angiotensin system. Diagnosis is based on increased serum aldosterone and urinary tetrahydroaldosterone. Cortisol metabolism, including urinary metabolites, is generally normal.

Glucocorticoid-suppressible aldosteronism is an autosomal dominant form of low-renin hypertension usually detected in children. It is diagnosed by elevated serum aldosterone and urinary excretion of tetrahydroaldosterone, 18-hydroxycortisol, and 18-oxo-tetrahydrocortisol.

Apparent mineralocorticoid excess (AME) is an autosomal recessive, severe form of hypertension usually detected in childhood. AME manifests with low renin and aldosterone and is diagnosed by low levels of urinary total cortisol metabolites, cortisone/cortisol ratio (or corresponding ratio of their metabolites), and tetrahydroaldosterone.

Hypoaldosteronism (Liddle's syndrome) manifests with hypertension and severe hypokalemia. Renin and aldosterone levels are often undetectable. Diagnosis is based on normal corticosteroid (cortisol) metabolism with low urinary tetrahydroaldosterone excretion.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Includes 31 adrenal and gonadal steroid metabolites
- Analytical sensitivity: 1.0 µg/L

Specimen Requirements

7 mL frozen aliquot of a 24-h urine

2.1 mL minimum

Refrigerate during collection.

Do not use preservatives.

Record total volume on vial and request form.

IA-2 Antibody 36177X

Clinical Use

- Assess risk of type 1 diabetes
- Predict onset of type 1 diabetes
- Diagnose new onset type 1 diabetes

Reference Range

Adults and children	<0.8 U/mL

Interpretive Information

- Patients at risk for developing type 1 diabetes
- Type 1 diabetic patients

Clinical Background

Type 1 diabetes mellitus is characterized by lymphocytic infiltration of islet cells and circulating autoantibodies against a variety of islet cell antigens (ICA). These include glutamic acid decarboxylase (GAD), insulin, IA-2, GM2-1, and others. Direct assays for autoantibodies to GAD-65, IA-2, and insulin are now available for detecting individuals with a genetic predisposition to type 1 diabetes.

A positive correlation exists between the IA-2 antibody concentration and the severity of the autoimmune process as well as between the number of positive autoantibodies (IA-2, GAD-65, and insulin) and the risk for type 1 diabetes. See Test Application and Interpretation section (Disorders of Carbohydrate Metabolism).

Method

- Radiobinding assay (RBA)
- Analytical sensitivity: 0.8 U/mL

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.5 mL minimum

IGF Binding Protein-1 (IGFBP-1) 36590X

Clinical Use

- Directly monitor insulin responsiveness
- Marker for insulinoma

Reference Range

	ng/mL
Adults	13-73
Children*	
5-9 y	20-105
10-14 y	10-70
15-18 y	10-40

*Data from: *J Clin Endocrinol Metab.* 1995; 80:2534-2542.

Interpretive Information

- ↑ Decreased insulin effect
- ↓ Increased insulin effect
- ↓ Insulinoma

Clinical Background

Insulin-like growth factors (IGF-I and IGF-II) are transported in serum bound to 1 of several binding proteins. One of the binding proteins, IGFBP-1, varies with glycemic conditions. When serum insulin is low (fasting), serum concentrations of IGFBP-1 quickly rise 4- to 6-fold. Conversely, infusion of glucose in normal subjects suppresses IGFBP-1 secretion.

Measuring IGFBP-1 is useful 1) for directly monitoring insulin responsiveness, 2) as a marker for insulin-producing tumors (insulinomas), 3) in diabetes mellitus for monitoring acute fluctuations in insulin action, 4) in diabetes mellitus for determining whether hyperglycemia is due to inadequate insulin dosage or to poor control of dietary intake in patients with chronically elevated HbA_{lc} or other indicators of poor long-term diabetic control, and 5) as a marker for insulin resistance associated with the metabolic syndrome.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 5 ng/mL
- Analytical specificity: no cross-reaction with IGFBP-2 or IGFBP-3

Specimen Requirements

1 mL frozen serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

IGF Binding Protein-2 (IGFBP-2) 37102X

Clinical Use

- Marker of growth hormone action
- Tumor marker for prostatic carcinoma

Reference Range

	ng/mL
1-9 y	69-480
10-17 y	50-326
18-49 y	55-240
>49 y	28-444

Interpretive Information

↑
- Chronic renal failure
- GH deficiency
- Leukemia
- Mesenchymal tumors (with hypoglycemia)
- Prostatic carcinoma

↓
- Acromegaly
- Severe liver disease

Clinical Background

Insulin-like growth factors (IGF-I, IGF-II) are transported in serum bound to several IGF binding proteins (IGFBP). These proteins bind both IGF-I and II with high affinity but do not bind insulin. The major function of these proteins is to transport IGFs within the circulation and out of the vascular compartment.

One of 6 IGF binding proteins, IGFBP-2 is present in very high concentrations during fetal development, progressively falling after birth to its lowest level between 18 and 35 years. The concentration of IGFBP-2 correlates inversely with changes in growth hormone (GH) secretion. GH administration will significantly suppress IGFBP-2 concentrations while increasing IGFBP-3 levels. Combined measurement of IGF-I, IGFBP-2, and IGFBP-3 allows direct assessment of GH action.

IGFBP-2 is also used as a tumor marker for prostatic carcinoma. Adult men with significant widespread prostatic carcinoma have IGFBP-2 levels 2 to 3 times greater than normal. In contrast, men with early-stage disease and normal prostate specific antigen (PSA) values showed an increase in IGFBP-2 levels of only 30%. This suggests that the test may be useful as an adjunct to PSA in the management of selected patients with prostatic carcinoma.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 0.56 ng/mL

Specimen Requirements

1 mL frozen serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

IGF Binding Protein-3 (IGFBP-3) 34458X

Clinical Use

- Differential diagnosis of GH deficiency and non-GH-deficient short children
- Assess nutritional status

Reference Range

	mg/L	mg/L
1-7 d		<0.7
8-15 d		0.5-1.4
16 d-1 y		0.7-3.6
2 y		0.8-3.9
3 y		0.9-4.3
4 y		1.0-4.7
5 y		1.1-5.2
6 y		1.3-5.6
7 y		1.4-6.1
8 y		1.6-6.5
9 y		1.8-7.1
10 y		2.1-7.7
11 y		2.4-8.4
12 y		2.7-8.9
13 y		3.1-9.5
14 y		3.3-10.0
15 y		3.5-10.0
16 y		3.4-9.5
17 y		3.2-8.7
18 y		3.1-7.9
19 y		2.9-7.3
20 y		2.9-7.2
21-30 y		3.4-7.8
31-40 y		3.4-7.0
41-50 y		3.3-6.7
51-60 y		3.4-6.9
61-70 y		3.0-6.6
71-80 y		2.5-5.7
81-85 y		2.2-4.5
>85 y	No primary data available; use 81-85 y/o range as a guideline	

Tanner stage	Male	Female
I	1.4-5.2	1.2-6.4
II	2.3-6.3	2.8-6.9
III	3.1-8.9	3.9-9.4
IV	3.7-8.7	3.3-8.1
V	2.6-8.6	2.7-9.1

Clinical Background

Insulin-like growth factor binding proteins bind IGF-I and IGF-II with high affinity but do not bind insulin. Of the 6 distinct IGF binding proteins structurally characterized at this time, IGFBP-3 has been shown to be the major carrier of the IGFs, transporting approximately 95% of circulating IGF-I and IGF-II.

IGFBP-3 is growth hormone (GH) responsive. Thus, levels are high in acromegaly and low in hypopituitarism, and levels increase in GH-deficient children after GH administration. IGFBP-2 levels, in contrast, decrease after GH administration. Thus, both assays and the ratios of IGF-I/IGFBP-2 and IGFBP-2/IGFBP-3 are useful as markers of GH action and for discriminating between growth hormone deficiency and short stature due to other etiologies in children. Other causes of short stature that result in reduced IGFBP-3 levels include poorly controlled diabetes. The IGFBP-3 assay is useful in assessing nutritional status, since IGFBP-3 decreases during both caloric and protein restriction.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.5 mg/L

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.5 mL minimum

SST red top unacceptable

Interpretive Information

↑
- Acromegaly

↓
- Hypopituitarism
- Isolated GH deficiency
- Poorly controlled diabetes
- Caloric and protein restriction

IGF-I

839X

Clinical Use

- Assess growth-related disorders
- Monitor growth hormone replacement therapy in adults with GH deficiency

Reference Range

	Male ng/mL	Female ng/mL
Adults		
19-30 y	126-382	138-410
31-40 y	106-255	126-291
41-50 y	86-220	88-249
51-60 y	87-225	92-190
61-70 y	75-228	87-178
71-80 y	31-187	25-171
81-88 y	68-157	31-162
>88 y	Not established	Not established
Children		
1-7 d	≤31	≤31
8-14 d	≤43	≤43
15 d-1 y	25-265	25-265
1-2 y	45-222	99-254
3-4 y	36-202	36-202
5-6 y	32-259	57-260
7-8 y	65-278	97-352
9-10 y	52-330	49-461
11-12 y	80-723	101-580
13-14 y	142-855	199-658
15-16 y	176-845	236-808
17-18 y	152-668	165-526
Tanner stages		
I	59-296	45-358
II	56-432	111-426
III	135-778	169-644
IV	230-855	297-627
V	181-789	142-868

Interpretive Information

- Acromegaly
- Gigantism

- Isolated GH deficiency
- Hypopituitarism
- Laron dwarfism
- Undernutrition

Clinical Background

Insulin-like growth factor I (IGF-I, somatomedin-C) is a protein involved in stimulating somatic growth; it is regulated principally by growth hormone (GH) and nutritional intake. IGF-I is transported in serum by several IGF-binding proteins. The most important are IGFBP-2 and IGFBP-3. These proteins help minimize fluctuations and maintain relatively high IGF-I serum levels.

Measuring IGF-I is useful in several growth-related disorders. Dwarfism caused by deficiency of growth hormone (hypopituitarism) results in decreased serum levels of IGF-I. In adults, a low IGF-I level in the presence of more than 3 pituitary hormone deficiencies obviates the need for a GH stimulation test. Acromegaly (growth hormone excess) results in elevated levels of IGF-I. IGF-I measurements are also helpful in assessing nutritional status; levels are reduced in undernutrition and restored with a proper diet. IGF-I has been utilized for dose titration in GH replacement therapy in adults with GH deficiency. The Endocrine Society consensus statement recommends achieving an IGF-I level that is within the sex and age adjusted reference range.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 25 ng/mL

Specimen Requirements

1 mL frozen serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

IGF-II (Insulin-Like Growth Factor II) 20707X

Clinical Use

- Evaluate non-islet-cell tumor hypoglycemia (NICTH)
- Assess growth factor status

Reference Range

Adults	ng/mL
18-30 y	546-1260
31-40 y	460-1240
41-50 y	414-1230
51-60 y	414-1248
Children	
0-4 y	Not established
5-9 y	754-1216
10-13 y	610-1217
14-17 y	649-1225

Interpretive Information

↑
- Mesenchymal tumors (with hypoglycemia)
- Hepatoma
- Acromegaly (variable)
- Wilms tumor

↓
- Infants under 1 year
- Growth hormone deficiency

Clinical Background

The somatomedins, insulin-like growth factors I and II (IGF-I and IGF-II), are structurally related to proinsulin and play an essential role in regulating cell proliferation and function. Their effects are mediated by way of IGF and insulin receptors. IGF-II is a neutral 7500-dalton polypeptide with 75% homology with IGF-I, and like IGF-I, it is produced in multiple tissues. It is more insulin-like in its action than IGF-I, and levels are less dependent on growth hormone. Plasma concentrations of IGF-II are approximately 3 times higher than IGF-I.

Non-islet-cell tumor hypoglycemia (NICTH) is a syndrome associated with low levels of insulin, IGF-I, and IGFBP-3 and increased secretion of "big" IGF-II (pro-IGF-II). The IGF-I/IGFBP-3 ratio is markedly increased. The preferred treatment is tumor removal, but growth hormone treatment can normalize the IGF-I/IGFBP-3 ratio and ameliorate the hypoglycemia.

Method

- Immunoradiometric assay (IRMA)
- Analytical sensitivity: 150 ng/mL

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.1 mL minimum

SST red top unacceptable

Overnight fasting is preferred.

Inhibin A 34472X

Clinical Use

- Prenatal risk assessment for Down syndrome

Reference Range

	pg/mL
Women	
Premenopausal	<98
Postmenopausal	<10
Men	<21

Interpretive Information

↑
- Granulosa cell tumors
- Down syndrome-affected pregnancies

↓
- Menopause

Clinical Background

Inhibin A is a glycoprotein produced by the placenta and by granulosa cells of the ovary, particularly during the late follicular and luteal phases of the menstrual cycle. The active form is a dimer consisting of an alpha subunit linked to a $beta_A$ subunit. Inhibin A has both endocrine (suppression of FSH by the pituitary) and paracrine (stimulation of thecal cell androgen synthesis) functions.

Measurement of inhibin B may be preferable to that of inhibin A for detection of recurrent granulosa cell tumors, studies of reproductive aging in females, and studies in males.

Method

- Enzyme-linked immunosorbent assay (ELISA)

Specimen Requirements

2 mL frozen serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Inhibin B

34445X

Clinical Use

- Detect recurrence of granulosa cell tumors
- Predict ovarian reserve and outcome of in vitro fertilization
- Marker of testicular function
- Differential diagnosis of cryptorchidism versus anorchia

Reference Range

	pg/mL	pg/mL
Men		<305
Women		
Premenopausal		<255
Postmenopausal		<30
Children	Males	Females
3-9 y	≤161	<30
10-13 y	42-339	≤92
14-17 y	68-300	≤139

Interpretive Information

- ↑ Granulosa cell tumors
- ↓ Decreased ovarian reserve/ menopause
- ↓ Anorchia

Clinical Background

Inhibin B is a glycoprotein produced by the Sertoli cells of the testis and the granulosa cells of the ovary, particularly during the early follicular phase of the menstrual cycle. The active form is a dimer consisting of an alpha subunit linked to a $beta_B$ subunit. Inhibin B suppresses pituitary synthesis of FSH and may also have additional paracrine roles within the ovary or testis. Measurement of inhibin B may be preferable to that of inhibin A for detection of recurrent granulosa cell tumors, studies of reproductive aging in females, and studies in males.

Method

- Enzyme-linked immunosorbent assay (ELISA)

Specimen Requirements

1 mL frozen serum (no additive red top tube); 0.5 mL minimum

Insulin Antibodies 36178X

Clinical Use

- Assess risk for type 1 diabetes
- Predict onset of type 1 diabetes
- Diagnose new onset type 1 diabetes

Reference Range

Adults and children	<1 U/mL

Interpretive Information

- Prediabetic (type 1) subjects
- Polyendocrine autoimmune syndromes
- Other autoimmune disease

Clinical Background

Type 1 diabetes is characterized by lymphocytic infiltration of pancreatic islet cells and autoantibodies against several islet cell antigens including glutamic acid decarboxylase (GAD-65), IA-2, and insulin. This assay is designed to assess the low titers of autoantibody in prediabetic subjects and other patients with autoimmune disorders who have not received exogenous insulin. For type 1 diabetes risk assessment, a combination of IA-2, GAD-65, and insulin autoantibodies increases the sensitivity.

Method

- Radiobinding assay (RBA)

Specimen Requirements

1 mL frozen serum (no additive red top tube); 0.2 mL minimum

SST red top unacceptable

Insulin, Free (Bioactive) 36700X

Clinical Use

- Monitor therapy in patients with insulin antibodies

Reference Range

Adults	4.0-20.0 µU/mL

Interpretive Information

- Following exogenous insulin injection
- Insulinoma
- Neisidioblastosis
- Some type 2 diabetic patients
- Insulin resistance

- Untreated type 1 diabetes mellitus

Clinical Background

The free insulin measurement is helpful in interpreting blood glucose concentration and its relationship to insulin injections in insulin-treated patients who have insulin antibodies. For instance, if a diabetic patient frequently becomes hypoglycemic at certain times of the day, concomitantly high free insulin levels would suggest the need to reduce the insulin dosage. In contrast, elevated glucose concentrations together with low free insulin levels indicate insufficient insulin for adequate control. Multiple, sequential, free insulin levels are also helpful for evaluating the rate and quantity of insulin absorption from subcutaneous injections.

The availability of C-peptide and free insulin measurements permits assessment of endogenous insulin secretion as well as the concentrations of circulating biologically active insulin (from all sources) in patients who have circulating insulin antibodies.

Method

- PEG precipitation, radioimmunoassay (RIA)
- Analytical sensitivity: 2.5 µU/mL
- Analytical specificity: detects free, bioavailable insulin; does not detect antibody-bound insulin; no cross-reactivity with C-peptide; cross-reacts with proinsulin

Specimen Requirements

1.8 mL refrigerated serum

0.6 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting required

Insulin, Total (Free and Bound) 36702X

Clinical Use

- Determine insulin dosage in patients with insulin-dependent diabetes and insulin antibodies

Reference Range

≤20.0 µU/mL

Interpretive Information

↑
- (With circulating insulin antibodies)
- Insulinoma
- Some type 2 diabetic patients
- Infantile hypoglycemia
- Hyperinsulinism

↓
- Untreated type 1 diabetes mellitus

Individuals with circulating insulin antibodies may demonstrate variable concentrations depending upon the level and binding capacity of the antibodies.

Clinical Background

The total insulin concentration includes free plus antibody-bound insulin. In patients without insulin antibody, total and free insulin concentrations are similar. In patients with insulin antibodies, total insulin levels are dependent on the binding capacity of the circulating endogenous insulin antibody and the availability of insulin to bind to the antibody sites. Total insulin is measured by eluting antibody-bound insulin with acid, precipitating the endogenous antibody, and measuring the total insulin by direct radioimmunoassay. Total insulin levels are useful in assessing the available insulin in serum, including antibody-bound insulin.

Serum insulin levels are useful in the differential diagnosis of fasting hypoglycemia (see Dynamic Test section) but must always be evaluated in the light of a simultaneous plasma glucose. With any cause of hyperglycemia, such as a meal, insulin is stimulated; with fasting or hypoglycemia, insulin is normally suppressed.

Method

- Acid treatment, PEG precipitation, radioimmunoassay (RIA)
- Analytical sensitivity: 2.5 µU/mL
- Analytical specificity: detects free and antibody-bound insulin; cross-reacts with proinsulin; no cross-reactivity with C-peptide

Specimen Requirements

1.8 mL refrigerated serum

0.6 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is required.

Invasive Trophoblast Antigen (ITA) (Pregnancy) 11303X

Clinical Use

- Early detection of pregnancy

Reference Range

<1 µg/L

Interpretive Information

- Pregnancy
- Trophoblastic tumors

Clinical Background

ITA is a hyperglycosylated form of human chorionic gonadotropin (hCG) secreted during embryonic implantation and trophoblast invasion of the uterine wall. ITA levels rise early in pregnancy, during which time almost 100% of hCG-related molecules is ITA. By 12 weeks of gestation, less than 2.9% of hCG-related molecules is ITA. Measurement of ITA rather than regular hCG therefore allows more dependable detection of pregnancy in the week after a missed menstrual period.

Method

- Electrochemiluminescence assay (ECLA)
- Analytical sensitivity: 0.5 µg/L
- Analytical specificity: <3.5% cross-reactivity with hCG; <1% cross-reactivity with other glycoprotein hormones

Specimen Requirements

2 mL room temperature serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Islet Cell Antibody (ICA) 36741X

Clinical Use

- Assess risk of type 1 diabetes
- Predict onset of type 1 diabetes
- Diagnose new onset type 1 diabetes

Reference Range

Antibody	Negative
Titer	<1.25 JDF Units

NOTE: End point titers are compared to a single international reference standard and values are reported in JDF (Juvenile Diabetes Foundation) units.

Interpretive Information

- Patients at risk for developing type 1 diabetes
- Type 1 diabetes

Clinical Background

Autoantibodies directed against pancreatic islet cells (islet cell antibodies, ICA) include glutamic acid decarboxylase (GAD), IA-2, insulin, GM2-1, and other cell surface proteins. Although ICA attacks all pancreatic islet cells, cell destruction appears specific for insulin-producing islet cells (beta cells), leading to insulin deficiency. ICA are present in the serum of patients during the prediabetic phase and predict development of type 1 disease.

Radiobinding assays are available for assessment of GAD-65, IA-2, and insulin autoantibodies and are more reliable as markers for the prediabetic state. This IFA assay measures a variety of antibodies, has limited sensitivity, and is only semi-quantitative.

Method

- Immunofluorescence assay
- Titer provided (at an additional charge) if antibody is present

Specimen Requirements

2.0 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

17-Ketosteroids (17-KS), Urine 15201X

Clinical Use

- Assess adrenocortical function

Reference Range

17-Ketosteroids	mg/24-h
Men	
17-20 y	9-22
>20 y	8-20
Women	
>16 y	6-15
Children	
<1 y	<1.0
1-4 y	<2.0
5-8 y	<3.0
9-12 y	3-10
13-16 y	5-12
Creatinine	**g/24-h**
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Cushing's syndrome
- Precocious puberty caused by adrenal hyperplasia
- Physiological stress (surgery, burns, infectious diseases)
- ACTH or gonadotropin stimulation

↓
- Addison's disease
- ACTH deficiency
- Corticosteroids, estrogen, oral contraceptives

Some medications may interfere with this test.

Clinical Background

17-Ketosteroids (17-KS) react with para-aminobenzoic acid to yield a colored product with an absorption maximum at 520 µM (the Zimmerman reaction). Adrenal androgens measured as 17-KS include dehydroepiandrosterone, androstenedione, etiocholanolone, androsterone, and their reduced metabolites. In men, about two-thirds of the 17-KS are secreted by the adrenal cortex, while in women, almost all are secreted by the adrenal cortex. Thus, in both sexes 17-KS excretion reflects adrenal androgen production.

Method

- Modified Zimmerman reaction
- Analytical sensitivity: 0.1 mg/L
- Creatinine concentration also reported

Specimen Requirements

20 mL refrigerated aliquot of a 24-h urine; 10 mL minimum

Collect urine with 10 g of boric acid (preferred); alternatively use 30 mL 6N HCL or 25 mL 50% acetic acid.

Record total volume on vial and request form.

17-Ketosteroids, Fractionated, 24-Hour Urine

4932X

Clinical Use

- Assess adrenal and gonadal androgen status

Reference Range

See next page

Interpretive Information

↑
- Hyperandrogenism
- Cushing's syndrome
- Adrenal hyperplasia

↓
- Adrenal insufficiency
- Chronic illness
- 5α-Reductase deficiency (decreased androsterone/etiocholanolone ratio)

Clinical Background

Androgens secreted by the adrenal gland or gonads are metabolized and excreted in the urine as 17-ketosteroid compounds. Fractionation of specific metabolites allows more specific assessment of androgen status than the older modified Zimmerman reaction assay. Detection by mass spectrometry further increases specificity as compared to capillary gas chromatography.

Androstenedione is metabolized to androsterone by 5α-reductase activity and to etiocholanolone by 5β-reductase activity. Both androsterone and etiocholanolone reflect overall levels of androstenedione (and, less directly, the levels of DHEA, testosterone, and 17-hydroxyprogesterone). The ratio of androsterone to etiocholanolone can be used as a relative index of 5α-reductase to 5β-reductase activity.

The 11-oxo and 11β-hydroxy metabolites of androsterone and etiocholanolone tend to reflect the patterns of the parent compounds. Urinary DHEA reflects overall serum DHEA levels. Pregnanetriol is a metabolite of 17-hydroxyprogesterone.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Creatinine concentration also reported

Specimen Requirements

5 mL frozen aliquot of a 24-h urine

2.6 mL minimum

Refrigerate during collection; do not use preservatives.

Continued

17-Ketosteroids, Fractionated, 24-Hour Urine *(continued)* 4932X

Reference Range

Androsterone	µg/24-h	µg/g creat
<1 y		3-225
1-4.9 y		4-378
5-9.9 y		16-830
10-13.9 y		102-1510
14-17.9 y		<2750
Men	320-5400	430-2500
Women	240-2300	150-2100
DHEA		
<1 y		≤100
1-4.9 y		≤100
5-9.9 y		≤100
10-13.9 y		5-316
14-17.9 y		15-732
Men	21-2170	24-1640
Women	21-2170	13-730
Etiocholanolone		
<1 y		6-29
1-4.9 y		2-261
5-9.9 y		18-465
10-13.9 y		98-1746
14-17.9 y		104-2006
Men	430-3300	280-2100
Women	245-2300	280-2100
11β-Hydroxyandrosterone		
<1 y		143-1180
1-4.9 y		23-673
5-9.9 y		44-677
10-13.9 y		100-802
14-17.9 y		68-1152
Men	130-900	180-850
Women	195-1500	180-850
11β-Hydroxyetiocholanolone		
<1 y		≤105
1-4.9 y		≤403
5-9.9 y		7-716
10-13.9 y		10-363
14-17.9 y		≤740
Adults	14-680	8-580

	µg/24-h	µg/g creat
11-Oxo-androsterone		
<6 mo		13-578
6-11.9 mo		1-200
1-4.9 y		3-69
5-9.9 y		6-118
10-13.9 y		4-104
14-17.9 y		8-106
Men	15-111	13-84
Women	8-87	13-84
11-Oxo-etiocholanolone		
<1 y		16-296
1-4.9 y		3-493
5-9.9 y		6-765
10-13.9 y		18-786
14-17.9 y		16-765
Adults	78-1165	68-870
Pregnanetriol		
<1 y		ND*
1-4.9 y		ND†
5-9.9 y		27-214
10-13.9 y		19-563
14-17.9 y		15-817
Men	71-1000	53-530
Women	47-790	53-530
Androsterone/Etiocholanolone Ratio		
<1 y		0.60-15.50
1-4.9 y		0.25-5.37
5-9.9 y		0.45-3.43
10-13.9 y		0.55-3.12
14-17.9 y		0.74-3.18
Creatinine		g/24-h
3-8 y		0.11-0.68
9-12 y		0.17-1.41
13-17 y		0.29-1.87
Adults		0.63-2.50

*Not determined; use random urine range (49-345 µg/g creat).

†Not determined; use random urine range (16-233 µg/g creat).

Leptin 37109X

Clinical Use

- Assess leptin control pathway

Reference Range

	Males ng/mL	Females ng/mL
Adults (BMI* 18-25)	1.2-9.5	4.1-25.0
Children†		
Prepubertal	1.6-10.8	1.7-10.6
Tanner II-III	2.1-11.6	2.6-11.5
Tanner IV-V	3.4-10.2	3.4-13.0

*BMI = body mass index = wt (kg)/ht (m^2)
†Pediatric reference ranges from *Clin Endocrinol.* 1997;46:727-733.

Interpretive Information

- Obesity
- Leptin receptor defect

- Under nutrition
- Leptin deficiency

Clinical Background

Leptin is an adipocyte-derived hormone essential for normal body weight regulation. Production is under neuroendocrine control such that blood levels vary directly with the amount of triglyceride stored in adipose tissue depots; there is a negative correlation between leptin and testosterone blood levels.

Leptin acts through specific receptors in the hypothalamus to modulate appetite and thermogenesis; increased levels suppress appetite and increase thermogenesis. Leptin deficiency leads to hypopituitarism including gonadotropin, growth hormone, and thyroid deficiencies. Gene mutations resulting in leptin deficiency or leptin receptor defects lead to massive obesity.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 0.5 ng/mL

Specimen Requirements

1.0 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

LH (Luteinizing Hormone) 615X

Clinical Use

- Diagnose gonadal function and pituitary disorders

Reference Range

	mIU/mL
Men	1.5-9.3
Women	
Follicular phase	1.9-12.5
Mid-cycle	8.7-76.3
Luteal phase	0.5-16.9
Postmenopausal	5.0-52.3

Interpretive Information

↑
- Primary hypogonadism
- Gonadotropin-secreting pituitary tumors
- Menopause

↓
- Hypothalamic GnRH deficiency
- Pituitary LH deficiency
- Ectopic steroid hormone production
- GnRH analog treatment

Clinical Background

Luteinizing hormone (LH, lutropin) is a glycoprotein produced by the anterior pituitary gland. Production is regulated by hypothalamic gonadotropin releasing hormone (GnRH) and feedback from gonadal steroid hormones. LH stimulates ovulation and ovarian steroid production (estrogen and progesterone) in the female. In the male, LH controls Leydig cell secretion of testosterone.

In females, LH concentrations are low during the follicular phase of the menstrual cycle, rise to a midcycle peak to cause ovulation, and, following ovulation, fall to levels lower than during the follicular phase. After menopause, LH concentrations rise to levels as high as or higher than those found in the midcycle peak; similar high levels are seen in castrated men.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.7 IU/L

This assay is appropriate for most purposes. The LH "third generation" assay (test code 36086X) is sensitive to 0.03 mIU/mL and is more appropriate for use in children.

Specimen Requirements

1.0 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

LH (Luteinizing Hormone), Pediatrics

36086X

Clinical Use

- Diagnose (and differential diagnosis of) gonadal dysfunction
- Monitor LH suppressive (Lupron®) therapy
- Applicable to pediatric samples

Reference Range

Children	Males mIU/mL	Females mIU/mL
1-7 y	≤0.10	≤0.45
8-9 y	≤0.44	≤3.36
10-11 y	≤2.28	≤5.65
12-14 y	0.31-5.29	≤11.00
15-17 y	0.15-5.33	≤15.80

Interpretive Information

- Primary hypogonadism
- Gonadotropin-secreting pituitary tumors

- Hypothalamic GnRH deficiency
- Pituitary LH deficiency

Clinical Background

Luteinizing hormone (LH, lutropin) is produced by the anterior pituitary gland. Production is regulated by hypothalamic gonadotropin releasing hormone (GnRH). LH stimulates ovulation and ovarian steroid hormone production in the female and testosterone secretion by the testes in the male.

GnRH and LH secretion are regulated by negative feedback systems, whereby reduced levels of gonadal hormones stimulate and increased levels inhibit circulating LH concentrations.

LH levels are useful in assessing disorders of gonadal function.

GnRH stimulation of LH is a useful provocative test in selected patients with delayed puberty and in confirming pituitary hypofunction in secondary hypogonadism.

Method

- Electrochemiluminescence assay (ECL)
- Analytical sensitivity: 0.03 mIU/mL
- Analytical specificity: 1.1% cross-reactivity with TSH; no cross-reactivity with FSH, GH, hCG, or prolactin

Specimen Requirements

0.5 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Macroprolactin 16122X

Clinical Use

- Rule out macroprolactinemia as a cause of increased circulating prolactin concentration

Reference Range

Prolactin, Total	ng/mL	ng/mL
Men		2.0-18.0
Women		
Non-pregnant		3.0-30.0
Pregnant		10.0-209.0
Post-menopausal		2.0-20.0
Children	Males	Females
Tanner I	≤10	3.6-12.0
Tanner II-III	≤6.1	2.6-18.0
Tanner IV-V	2.8-11.0	3.2-20.0
Prolactin, Monomeric		*
Prolactin, % Monomeric		≥50%†
Macroprolactin		<50%†

*ng/mL monomeric prolactin still above total prolactin reference range suggests hyperprolactinemia.
†% monomeric prolactin <50% (macroprolactin ≥50%) indicates presence of macroprolactinemia.

Interpretive Information

Macroprolactin

↑
- Macroprolactinemia

Monomeric Prolactin

↑
- Hypothalamic or pituitary tumor
- Stress (physical and emotional)
- Antidepressants
- Breast-feeding

↓
- Dopamine agonists (eg, bromocriptine, cabergoline, pergolide)

Clinical Background

Prolactin (PRL), a 23 kd peptide secreted by the anterior pituitary gland, mostly circulates in a monomeric, biologically active, free form. Two other forms have been identified: big PRL (40-60 kd) and big-big PRL (>100 kd). Big-big PRL (macroprolactin) is most frequently a complex of anti-PRL IgG and PRL and is believed to be biologically inactive. Unlike hyperprolactinemia, macroprolactinemia comprised of big PRL and/or big-big PRL does not appear to be associated with increased incidence of pituitary adenomas. Since most PRL assays cross-react with macroprolactin in varying degrees, they may give artificially high PRL results in the presence of macroprolactinemia, leading to an incorrect diagnosis of hyperprolactinemia.

In this test, the PRL level is determined from 2 aliquots, 1 of which was treated with polyethylene glycol (PEG) to remove high molecular weight isoforms, including macroprolactin, and 1 with no such treatment. If the PRL obtained from the treated sample (ie, monomeric PRL) normalizes and is <50% of that in the untreated sample, macroprolactin is present. A monomeric PRL that is still above normal may be due to co-existence of macroprolactinemia with hyperprolactinemia and necessitates ruling out a pituitary adenoma.

Method

- Immunochemiluminometric assay (ICMA) with and without PEG precipitation
- Analytical sensitivity: 1.0 ng/mL

Specimen Requirements

1.0 mL frozen serum

0.4 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting preferred

MEN 2 and FMTC Mutations, Exons 10, 11, and 13-16

36587X

Clinical Use

- Screen at-risk individuals for MEN 2 and FMTC
- Diagnose MEN 2 and FMTC
- Differentiate between familial and sporadic MTC

Reference Range

Negative (no mutations detected)

Interpretive Information

Mutation present

- FMTC and/or MEN 2A
- MEN 2B

Clinical Background

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant syndrome associated with a high risk of medullary thyroid carcinoma (MTC). The MEN 2A subtype is associated with hyperparathyroidism, and both MEN 2A and MEN 2B subtypes are associated with an increased risk for pheochromocytoma. MEN 2B is further characterized by mucosal neuromas on the lips and tongue and in the gastrointestinal tract.

Over 95% of MEN 2A cases and over 85% of familial MTC (FMTC) cases have a germline mutation in 1 of 5 conserved cysteine residues encoded in exon 10 or 11 of the RET proto-oncogene. A point mutation in exon 16 is present in over 95% of MEN 2B cases. Other mutations in these exons and in exons 13, 14, and 15 have also been associated with MEN 2.

Once the mutation in an affected family member has been identified, testing of first-degree relatives allows presymptomatic identification of those at risk for MEN 2 and prophylactic treatment. Correlation between specific mutations and aggressiveness of MTC may aid in appropriately timing prophylactic thyroidectomy and pheochromocytoma screening. Genetic testing is recommended for all individuals with MTC since RET mutations have been detected in 1% to 20% of those who appear to have sporadic MTC (ie, those without a known family history of MEN 2).

Method

- Polymerase chain reaction (PCR) and semi-automated DNA sequencing
- Analytical specificity: mutations in exons 10, 11, 13, 14, 15, and 16 of the RET proto-oncogene

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube. Alternatively, submit amniotic fluid, bone marrow, tissue biopsy (eg, dissected CVS) or extracted DNA.

Metanephrines, Fractionated, Plasma 19548X

Clinical Use

- Diagnose pheochromocytoma

Reference Range

	pg/mL
Metanephrine	≤57
Normetanephrine	≤148
Metanephrines, Total	≤205

Interpretive Information

- Pheochromocytoma
- Neuroblastoma
- Stress

Clinical Background

Normetanephrine and metanephrine are the extra-neuronal catechol-o-methyl transferase (COMT) metabolites of the catecholamines norepinephrine and epinephrine, respectively. Measurement of plasma metanephrines is more sensitive for the detection of pheochromocytoma, but may be less specific, than measurement of catecholamines. Proper interpretation of results requires awareness of recent medication/drug history (eg, antihypertensive agents, alcohol, cocaine) and other pre-analytical factors (eg, stress, severe congestive heart failure, myocardial infarction) that influence release of catecholamines and metanephrines.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 25 pg/mL
- Analytical specificity: cross-reacts 0.08% with acetaminophen and 0.03% with 3-hydroxy tyramine (dopamine)

Specimen Requirements

2.5 mL refrigerated EDTA plasma

1.5 mL minimum

Patient preparation: abstain from alcohol, coffee, tea, tobacco, strenuous exercise, and, if possible, medication for 3 days prior to collection. Overnight fasting is preferred. Patient should be seated in a relaxed condition before collection of sample.

Metanephrines, Fractionated, Urine

14962X
14961X

Clinical Use

- Diagnose pheochromocytoma

Reference Range

	14962X	*14961X*
Metanephrine	µg/24-h	µg/g creat
3 mo-4 y	25-117	Not established
5-9 y	11-139	106-527
10-13 y	51-275	34-357
14-17 y	40-189	24-302
18-29 y	25-222	39-146
30-39 y	36-190	32-134
40-49 y	58-203	33-192
≥50 y	90-315	21-153
Normetanephrine		
3 mo-4 y	54-249	Not established
5-9 y	31-398	149-781
10-13 y	67-503	38-523
14-17 y	69-531	14-302
18-29 y	40-412	91-365
30-39 y	35-482	67-390
40-49 y	88-649	85-514
≥50 y	122-676	108-524
Metanephrines, Total		
3 mo-4 y	79-345	Not established
5-9 y	49-408	255-1167
10-13 y	110-714	86-845
14-17 y	107-741	39-578
18-29 y	94-604	156-442
30-39 y	115-695	94-445
40-49 y	182-739	155-608
≥50 y	224-832	149-603
Creatinine		mg/dL
0-6 mo		2-32
7-11 mo		2-36
1-2 y		2-128
3-8 y		2-149
9-12 y		2-183
>12 y		
Males		20-370
Females		20-320

Clinical Background

A diagnosis of pheochromocytoma can be confirmed by measuring urinary levels of the catecholamines and the catecholamine metabolite vanillyl-mandelic acid. Urinary metanephrine determinations are considered the most accurate single screening method for pheochromocytoma. Quantitative assessment using 24-hour urine collection or semiquantitation relative to creatinine excretion is recommended.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 5 µg/L

Specimen Requirements

24-Hour urine (14962X)
Random urine (14961X)

5 mL room temperature aliquot of urine; 1.5 mL minimum

After urine collection, add 6N HCl to maintain a pH below 3. Record patient's age and total volume (24-h sample only) on vial and request form.

Patient preparation: abstain from medication, tobacco, tea, and coffee for 3 days prior to collection.

Interpretive Information

- Pheochromocytoma
- Neuroblastoma
- Stress

Microalbumin, Intact, Urine

17105X
17102X

Clinical Use

- Early detection of diabetic nephropathy
- Therapeutic monitoring of patients with nephropathy
- Routine management of patients with diabetes (ADA-recommended)

Reference Range

Microalbumin (adults)	
24-Hour urine	<30 mg/24-h
Random urine	<30 mg/g creat
Creatinine	g/24 h
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
Adults	0.63-2.50
	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

Elevated levels are diagnostic only after confirmation on 2 to 3 specimens collected within a 3-6 month period.

Interpretive Information

	mg/24-h	mg/g creat
Normal (non-diabetic)	<30	<30
Micro-albuminuria	30-299	30-299
Clinical albuminuria	≥300	≥300

Reference: American Diabetes Association (ADA). *Diabetes Care.* 2006; 29(Suppl 1): S4-S42.

Clinical Background

Renal impairment (nephropathy) is common in both type 1 and type 2 diabetes. Slight increases in urinary excretion of albumin (micro-albuminuria = 30 to 299 mg/24 hour) can predict clinical nephropathy in patients with diabetes. In contrast, clinical albuminuria (≥300 mg/24 hour) usually indicates failing renal function. Microalbuminuria is an early indication of impending renal failure, which is potentially reversible by strict glycemic control and antihypertensive therapy.

This high performance liquid chromatography (HPLC) method detects both immunochemically reactive albumin and unreactive intact albumin. Immunochemically unreactive intact albumin is not detected by immunoassays. The HPLC method can therefore detect more cases of microalbuminuria than dipstick or immunoassay methods.

Method

- HPLC
- Analytical sensitivity: 3 mg/mL
- Not evaluated in children <18 y
- Not evaluated in those with peripheral vascular disease
- Creatinine concentration also reported

Specimen Requirements

24-Hour urine (17105X)
Random urine (17102X)

1 mL frozen aliquot of a 24-h urine

0.4 mL minimum

Do not use a preservative; avoid glass containers. Record total volume on vial and request form (24-h sample only).

Nephrogenic Diabetes Insipidus (Autosomal) Mutations 15028X

Clinical Use

- Differentiate inherited NDI from acquired NDI
- Screen for autosomal NDI carrier status in at-risk individuals

Reference Range

Negative (no mutations detected)

Interpretive Information

Mutation present

- Nephrogenic diabetes insipidus (affected or carrier)

Clinical Background

Nephrogenic diabetes insipidus (NDI) is characterized by inability of the kidneys to concentrate urine despite the presence of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH). This insensitivity to AVP results in polyuria, polydipsia, low urinary specific gravity, and high risk of severe dehydration, especially in affected infants.

Although acquired NDI is more common than inherited disease, genetic forms also occur. They can be transmitted in X-linked recessive, autosomal recessive, or autosomal dominant patterns. X-linked NDI, caused by mutations in the *AVPR2*, is the most common genetic cause of NDI. Most cases of autosomal NDI have a recessive mode of inheritance, but about 10% of them (1% of all inherited NDI) result from mutations with dominant expression.

Insertion of the aquaporin 2 (AQP2) protein into the luminal membrane of collecting duct cells is the final step in the antidiuretic action of AVP, providing specific channels that increase water permeability of the membrane. Mutations disrupting the *AQP2* gene on chromosome 12 alter the amino acid sequence of the AQP2 protein and cause about 10% of familial NDI. Since about 25 different mutations have been identified in the *AQP2* gene, gene sequencing is the method of choice for detecting *AQP2* mutations. After identification of a mutation in an affected individual, genetic testing can be used to evaluate other family members.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Analytical specificity: mutations in 4 exons of the *AQP2* gene

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube

Nephrogenic Diabetes Insipidus (X-Linked) Mutations

15034X

Clinical Use

- Differentiate inherited NDI from acquired NDI
- Screen for X-linked NDI carrier status in at-risk females

Reference Range

Negative (no mutations detected)

Interpretive Information

Mutation present

- Nephrogenic diabetes insipidus (affected or carrier)

Clinical Background

Nephrogenic diabetes insipidus (NDI) is characterized by inability of the kidneys to concentrate urine despite the presence of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH). This insensitivity to AVP results in polyuria, polydipsia, low urinary specific gravity, and high risk of severe dehydration, especially in affected infants. Although acquired NDI is more common than inherited disease, genetic forms also occur. They can be transmitted in X-linked recessive, autosomal recessive, or autosomal dominant patterns. The X-linked form is the most common cause of inherited NDI.

When functional AVP receptor 2 (AVPR2) protein is not present on the basolateral membrane of collecting duct cells, the kidney is not able to respond to the antidiuretic action of AVP. Mutations disrupting the *AVPR2* gene on the X chromosome cause about 90% of inherited NDI. Because the *AVPR2* gene is on the X chromosome, the disorder is most common in males, but some female heterozygotes also have symptoms. Since about 180 different mutations have been identified in the *AVPR2* gene, gene sequencing is the method of choice for detecting *AVPR2* mutations. After identification of a mutation in an affected individual, genetic testing can be used to evaluate other family members.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Analytical specificity: mutations in the X-linked *AVPR2* gene

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect blood in a lavender-top (EDTA) or yellow-top (ACD solution B) tube.

Osmolality, Random Urine 678X

Clinical Use

- Diagnose and manage fluid and electrolyte disorders

Reference Range

50-1200 mOsm/kg

Interpretive Information

- Dehydration
- Syndrome of inappropriate ADH secretion (SIADH)
- Liver disease
- Heart disease

- Overhydration
- Hypokalemia
- Diabetes insipidus

Clinical Background

Osmolality is a measurement of the amount of nonelectrolyte substances dissolved per kilogram of pure water. Urine osmolality is a measure of solute concentration in the urine and reflects the concentrating ability of the kidney. The major factors regulating urine osmolality are vasopressin (antidiuretic hormone) and the urine-concentrating apparatus of the kidney, which responds to vasopressin. Consequently, pituitary and renal disorders, as well as cardiovascular dysfunction impairing renal blood flow, can impair physiological regulation of urine osmolality.

Method

- Freezing point depression

Specimen Requirements

1 mL refrigerated, random urine

0.2 mL minimum

Do not use a preservative.

Osmolality, Serum 677X

Clinical Use

- Assess fluid and electrolyte balance

Reference Range

278-305 mOsm/kg

Interpretive Information

↑
- Dehydration
- Hyperglycemia
- Hypernatremia
- Alcohol poisoning
- Diabetes insipidus

↓
- Overhydration
- Hyponatremia
- Syndrome of inappropriate ADH secretion (SIADH)

Clinical Background

Osmolality is a measurement of the amount of nonelectrolyte substances dissolved per kilogram of pure water. Serum osmolality is an index of solute concentration in the blood. It is precisely controlled by hypothalamic-posterior pituitary regulation of vasopressin secretion, leading to modulation of water excretion by the kidneys. Measurement of serum osmolality is helpful in assessing effectiveness or dysfunction of this system.

Method

- Freezing point depression

Specimen Requirements

1 mL room temperature serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Osteocalcin (Bone Gla Protein, BGP) 5586X

Clinical Use

- Determine efficacy of therapy in osteoporosis and metastatic bone disease
- Useful marker of bone formation and remodeling

Reference Range

	ng/mL
Men	11.3-35.4
Women	7.2-27.9
Children	
6-9 y	40.2-108.0
10-13 y	35.8-165.5
14-17 y	
Males	27.8-194.1
Females	16.3-68.7

Interpretive Information

↑
- Primary hyperparathyroidism
- Secondary osteosarcoma
- Healing bone fractures
- Hyperthyroidism
- Paget's disease
- Therapeutic agents such as phenytoin and 1,25-dihydroxyvitamin D

↓
- Hypoparathyroidism
- Cushing's syndrome

Clinical Background

Osteocalcin is a 6-kd protein constituting 1% to 2% of total bone protein. It contains 3 gamma-carboxyglutamic acid (Gla) residues that bind hydroxylapatite. Osteocalcin is produced exclusively by bone osteoblasts, and production is dependent on 1,25-dihydroxyvitamin D, vitamin K, and vitamin C. Measurement provides a specific biochemical index of bone activity. As assessed by bone histomorphometry, serum osteocalcin levels correlate with bone formation and not directly with bone resorption. Serum osteocalcin is an important bone formation marker.

Method

- Immunoradiometric assay (IRMA)
- Analytical sensitivity: 2.0 ng/mL

Specimen Requirements

1 mL frozen serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

Avoid hemolysis and lipemia.

Pancreatic Polypeptide 4789X

Clinical Use

- Assess pancreatic tumor burden
- Assist in early diagnosis of pancreatic tumors
- Monitor pancreatic carcinoma therapy
- Predict recurrence of pancreatic tumors

Reference Range

Adults	pg/mL
18-29 y	≤480
30-39 y	70-400
40-49 y	70-430
50-62 y	100-780
>62 y	Not established
Children	
Cord blood	
Term infants*	≤163
Preterm infants*	≤180
Term infants-6 d*	≤276
1 mo-2 y*	≤644
3-9 y	≤519
10-13 y	≤361
14-17 y	≤297

*Data from *Reprod Nutr Dev.* 1990;30:65-70, *Materia Medica Polona.* 1989;21:38-42, and *Acta Paediatr Scand.* 1980;69:211-214.

Interpretive Information

- VIPoma
- Insulinoma
- Medullary thyroid carcinoma
- Zollinger-Ellison syndrome

Clinical Background

Pancreatic polypeptide (PP) is a 32-amino acid peptide that is produced primarily by the endocrine cells of the pancreas; it is also present in extracts of gastric mucosa. The physiological role of PP is not clearly defined. Secretion is influenced by hypoglycemia, pentagastrin injection, and ingestion of protein meals. Plasma concentrations normally change with age, type of food ingestion, and time of blood collection.

PP concentrations are used in the management of PP-producing tumors. High plasma levels have been observed in some patients with pancreatic endocrine tumors (VIPomas and insulinomas), multiple endocrine neoplasia syndrome type 1 (MEN 1), medullary thyroid carcinoma, and Zollinger-Ellison syndrome.

In patients with MEN 1, a PP level greater than 3 times the age-matched normal mean is suggestive of an islet cell tumor.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 50 pg/mL
- Analytical specificity: no cross-reaction with glucagon or insulin

Specimen Requirements

2 mL refrigerated EDTA plasma

0.6 mL minimum

Overnight fasting is preferred.

Pheochromocytoma Gene Mutations

19568X, 19567X, 19571X

Clinical Use

- Diagnose or exclude pheochromocytoma or paraganglioma caused by mutations in the *SDHB* (test code ***19568X***), *SDHD* (test code ***19567X***), and *VHL* (test code ***19571X***) genes
- Screen individuals who have a family history of pheochromocytoma and/or paraganglioma
- Assist in treatment selection for patients with a gene mutation

Reference Range

Negative (no mutations detected)

Interpretive Information

SDHB mutation present

- Abdominal or thoracic pheochromocytoma
- Paraganglioma type 4

SDHD mutation present

- Head or neck pheochromocytoma
- Paraganglioma type 1

VHL mutation present

- Adrenal or thoracic pheochromocytoma
- von Hippel-Lindau syndrome

Clinical Background

Pheochromocytomas are catecholamine-producing tumors that arise from chromaffin cells, the largest compact collection of which is in the adrenal medulla. Tumors that arise within the adrenal medulla are referred to as pheochromocytomas, whereas those arising outside the adrenal are called paragangliomas or extraadrenal pheochromocytomas. These tumors are characterized by hypertension and elevated levels of catecholamines and should be differentiated from ganglioneuromas. Although affecting only a small percentage of patients with hypertension, pheochromocytomas are usually curable; however, they are often fatal if left untreated.

Pheochromocytoma is familial in up to 25% of affected individuals; age of onset is <30 years. The genes involved include *NF-1; RET* (see MEN and FMTC Mutations, test code ***36587X***); succinate dehydrogenase subunits B (*SDHB*), C (*SDHC*), and D (*SDHD*); and *VHL*. Pheochromocytoma causing *NF-1* and *SDHC* mutations are rare.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Analytical specificity: *SDHB* (8 exons) (test code ***19568X***), *SDHD* (4 exons) (test code ***19567X***), and *VHL* (3 exons) (test code ***19571X***); large deletions and deep intronic mutations affecting mRNA splicing are not detected

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect in lavender-top (EDTA) tube

Plasma Renin Activity (PRA) 10537X

Clinical Use

- Assess renin-aldosterone axis
- Differential diagnosis of hypertension

Reference Range

Adults*	ng/mL/h	ng/mL/h
Supine		0.3-3.0
Upright		0.4-8.8
Upright/sitting		0.65-5.0
Children†	**Supine**	**Upright**
3-12 mo	≤15.0	–
1-3 y	≤10.0	–
4-6 y	≤7.5	≤15.0
7-9 y	≤5.9	≤17.0
10-12 y	≤5.3	≤16.0
13-15 y	≤4.4	≤16.0
Clinical Cut-off Values††		
Sodium/volume hypertension likely		<0.65
Primary aldosteronism possible§		<0.65
Renin-mediated hypertension likely		≥0.65
Renovascular hypertension possible		>1.5
Renovascular hypertension more likely		>10.0

*In apparently healthy, non-hypertensive, non-medicated, non-pregnant individuals on unrestricted diets.
†Pediatric data from *J Pediatr.* 1976;89:256; *Pediat Res.* 1979;13:817; and *Eur J Pediatr.* 1994;153:284.
††In ambulatory, briefly seated hypertensive patients.
§To be used in conjunction with aldosterone concentration; this is a rare condition.

Interpretive Information

↑
- Renal hypertension
- Addison's disease
- Secondary hypoaldosteronism
- Medications (eg, diuretics, ACE inhibitors)

↓
- Hyporeninemic hypoaldosteronism
- Primary aldosteronism
- Essential hypertension (1/3rd of cases; PRA <0.65 ng/mL/h)

Clinical Background

Renin is a proteolytic enzyme produced by the kidney. Secretion is modulated by changes in renal blood flow. Erect posture, sodium depletion, hemorrhage, and low cardiac output all increase renin secretion by reducing flow.

The measurement of plasma renin activity (PRA) is useful in evaluating hypertension. Primary hyperaldosteronism is associated with sodium retention, increased blood volume, increased renal blood flow, and low PRA. Thus, a normal or high PRA rules out primary aldosteronism. Conversely, a normal or low PRA helps rule out renal hypertension. Additionally, an elevated PRA may indicate renovascular hypertension due to renal artery stenosis.

The interpretation of PRA values is facilitated by measurements of aldosterone and serum potassium levels.

	Renin	Aldosterone	Potassium
1° aldosteronism	Low	High	Low
Hyporeninemic hypoaldosteronism	Low	Low	High

Method

- Angiotensin I generation, radioimmunoassay (RIA)
- Analytical sensitivity: 0.37 ng/mL/h

Specimen Requirements

3 mL frozen EDTA plasma

0.4 mL minimum

Avoid refrigerated temperatures. When submitting catheterization studies, retain a portion of each sample at the referring laboratory.

Patient preparation: moderate sodium diet; ambulatory for 30 minutes; no medications, preferably for 3 weeks prior to sample collection.

Pregnanetriol, Urine 738X

Clinical Use

- Diagnose congenital adrenal hyperplasia (CAH) (21-hydroxylase deficiency)
- Therapeutic monitoring of patients with 21-hydroxylase deficiency

Reference Range

Pregnanetriol	µg/24-h
Males	71-1000
Females	47-790
	µg/g creat
Males and females	53-530
Creatinine	g/24-h
3-8 y	0.11-68
9-12 y	0.17-1.41
13-17 y	0.29-1.87
≥18 y	0.63-2.50

Interpretive Information

- ↑ CAH caused by 21-hydroxylase enzyme deficiency
- Stein-Levinthal syndrome
- Adrenal and ovarian tumors
- ↓ 17-hydroxylase deficiency syndrome (rare)
- Ovarian failure post puberty

Clinical Background

Pregnanetriol is a metabolite of the adrenal steroid precursor, 17-hydroxyprogesterone. 21-Hydroxylase catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol. However, when there is decreased 21-hydroxylase activity, 17-hydroxyprogesterone accumulates in the blood and its breakdown product, pregnanetriol, accumulates in urine.

17-Hydroxyprogesterone is also a normal secretory product of the ovary. There is a significant increase in secretion at midcycle, coinciding with the LH peak, and an increase during the luteal phase, correlating with increases in estradiol and progesterone secretion. Pregnanetriol subsequently increases. Thus, ovarian disorders can be associated with abnormal pregnanetriol levels as well.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Creatinine concentration also reported

Specimen Requirements

5.0 mL frozen aliquot of a 24-h urine collection; 2.1 mL minimum

Refrigerate during collection; do not use preservatives.

Pregnenolone 31493X

Clinical Use

- Diagnose 3β-hydroxysteroid dehydrogenase deficiency
- Diagnose adrenal virilizing tumors

Related Assays

- 17-Hydroxypregnenolone
- DHEA
- DHEA-sulfate

Reference Range

	ng/dL	ng/dL
Men		10-200
Women		10-230
Children		
ACTH stimulation	Baseline	60 min
<1 y	10-140	49-360
1-5 y	10-48	34-135
6-12 y	15-45	39-105
Tanner II-III		
Males	10-45	58-110
Females	15-84	33-140
Tanner IV-V		
Males	11-50	37-150
Females	20-77	91-220

Pediatric data from *J Clin Endocrinol Metab.* 1992;75:1491-1496.

Interpretive Information

- Congenital adrenal hyperplasia
- Adrenal virilizing tumor
- Cushing's disease
- Normal children and adults after ACTH administration

Clinical Background

Normal steroid-producing cells of the adrenal glands and gonads synthesize various steroids from circulating cholesterol ester. This results in small amounts of precursors such as pregnenolone, a $\triangle^5$ C-21 steroid, leaking into the circulation. Most of the circulating pregnenolone is from the adrenal cortex, and levels are modestly increased after ACTH administration in normal children and adults.

This assay is useful in diagnosing 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency, an unusual form of congenital adrenal hyperplasia associated with blocked cortisol synthesis and increased levels of $\triangle^5$ steroids. After diagnosis of 3β-HSD deficiency, the test can evaluate glucocorticoid replacement therapy. The assay is also helpful in suggesting the presence of an adrenal virilizing tumor, since almost all of these tumors secrete large amounts of DHEA-sulfate and pregnenolone.

Pregnenolone levels are moderately increased in Cushing's disease due to ACTH-secreting pituitary adenoma or ectopic causes.

Method

- Extraction, chromatography, radioimmunoassay (RIA)
- Analytical sensitivity: 10 ng/dL

Specimen Requirements

4 mL refrigerated serum

1.1 mL minimum

No additive red top preferred

SST red top acceptable

Progesterone 17183X

Clinical Use

- Assess ovarian function
- Assess abnormal pregnancy
- Detect progesterone-secreting tumors

Reference Range

Men	ng/mL	ng/mL
18-29		≤0.3
30-39		≤0.2
40-49		≤0.2
50-59		≤0.2
Women		
Early follicular phase		≤0.6
Late follicular phase		≤14.5
Mid-cycle		≤16.1
Luteal phase		≤31.4
Postmenopausal		≤0.2
Children	Males	Females
5-9 y	≤0.7	≤0.6
10-13 y	≤1.2	≤10.2
14-17 y	≤0.8	≤11.9

Interpretive Information

- Ovarian tumor
- Pregnancy
- Adrenal tumor
- Selected steroid hormone biosynthetic defects

- Amenorrhea
- Fetal death
- Threatened abortion
- Gonadal agenesis

Clinical Background

In the cycling female, the primary source of progesterone is the corpus luteum. After ovulation, a corpus luteum is formed, and there is a dramatic rise in progesterone levels that persists for about 2 weeks. If pregnancy occurs, corpus luteum survival is prolonged, and progesterone is necessary to maintain pregnancy. As pregnancy advances, progesterone is secreted by the placenta. Decreased levels of progesterone are seen in the short and inadequate luteal phase and in the first trimester of abnormal pregnancies.

Progesterone is secreted by the adrenal gland in adult males and in children. In addition, high levels of progesterone can indicate tumors of the adrenals or ovaries.

Method

- Liquid chromatography, tandem mass spectrometry (LC/MS/MS)
- Includes online extraction, HPLC separation, and MS/MS
- Analytical sensitivity: 0.1 ng/mL

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.25 mL minimum

SST red top unacceptable

An early-morning specimen is preferred.

Specify age, sex, and menopausal status on test request form.

Proinsulin 760X

Clinical Use

- Diagnose insulinomas
- Monitor therapy in patients with insulinomas

Reference Range

	pmol/L
Adults	≤18.8

Interpretive Information

↑
- Insulinoma
- Severe hypoglycemic hypoinsulinemia
- Chronic renal failure
- Hyperthyroidism
- Familial hyperproinsulinemia

Clinical Background

Proinsulin is produced in the beta islet cells of the pancreas and is cleaved into insulin and C-peptide prior to release into the circulation. Normally, a small amount of proinsulin (2% to 3%) escapes conversion and is secreted along with the insulin during beta cell stimulation. Proinsulin has a slight hypoglycemic action, and severely hypoglycemic hyperinsulinemic patients have shown that up to 40% of their serum immunoreactive insulin is proinsulin-like material.

The clinical disorder that most consistently results in elevated serum proinsulin is the insulinoma, a benign or malignant islet cell tumor of the pancreas. Increased levels of proinsulin have also been reported in patients with chronic renal failure, hyperthyroidism, and familial hyperinsulinemia.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 5.0 pmol/L

Specimen Requirements

1 mL frozen serum

0.8 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is required.

Prolactin 746X

Clinical Use

- Diagnose and manage pituitary adenomas
- Differential diagnosis of male and female hypogonadism

Reference Range

	ng/mL
Men	2.0-18.0
Women	
Nonpregnant	3.0-30.0
Pregnant	10.0-209.0
Postmenopausal	2.0-20.0
Children	
Males	
Tanner I	≤10
Tanner II-III	≤6.1
Tanner IV-V	2.8-11.0
Females	
Tanner I	3.6-12.0
Tanner II-III	2.6-18.0
Tanner IV-V	3.2-20.0

Interpretive Information

- Hypothalamic or pituitary tumor
- Stress (physical and emotional)
- Antidepressants
- Breast-feeding

- Bromocriptine (dopamine agonist)

Clinical Background

Prolactin is a protein hormone secreted by the anterior pituitary gland and the placenta. It may modulate the number of follicles developing in the follicular phase of each menstrual cycle. During and following pregnancy, prolactin, in association with other hormones, stimulates breast development and milk production.

Prolactin secretion is stimulated by sleep, stress (physical and emotional), and the hypothalamic hormone, thyrotropin releasing hormone (TRH). Prolactin secretion is decreased by dopamine and dopamine analogs such as bromocriptine.

Hypersecretion of prolactin can be caused by pituitary tumors, hypothalamic disease, breast or chest wall stimulation, hypothyroidism, renal failure, acute exercise, stress, eating, and several medications (eg, phenothiazines, metoclopramide). Hyperprolactinemia inhibits gonadotropin secretion and can produce hypogonadism in men and women with accompanying low or inappropriately "low normal" LH and FSH levels.

Method

- Immunochemiluminometric assay (ICMA), ADVIA Centaur®'
- Analytical sensitivity: 1.0 ng/mL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Overnight fasting is preferred.

PTH Antibody 36578X

Clinical Use

- Assess possibility of antibody interference with PTH measurements

Reference Range

Negative

Interpretive Information

- Autoimmune disease
- PTH therapy for osteoporosis

Clinical Background

Parathyroid hormone (PTH) autoantibodies may lead to misleading results when measuring PTH by immunoassay methods. Such autoantibodies are rare but may be seen in autoimmune disease states and in patients who are receiving PTH therapy for osteoporosis.

Method

- Radiobinding assay (RBA)

Specimen Requirements

1 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

PTH, Intact and Calcium

8837X
36736X

Clinical Use

- Discriminate primary hyperparathyroidism from tumor hypercalcemia
- Diagnose hypoparathyroidism
- Monitor severity of secondary hyperparathyroidism in chronic renal failure

Reference Range

PTH, Intact	pg/mL
Adults	10-65
Children	
6-9 y	9-59
10-13 y	11-74
14-17 y	9-69
Calcium	mg/dL
Adults	8.8-10.1
Ionized Calcium	mg/dL
Adults	4.6-5.4
Children	
8 mo-10 y	4.9-5.4
11-17 y	4.8-5.3

Pediatric data from *J Pediatr.* 1989;114:952-956 and *Eur J Pediatr.* 1991;150:464-467.

Interpretive Information

- Primary hyperparathyroidism
- Secondary hyperparathyroidism
- Renal failure
- Pseudohypoparathyroidism

- Hypoparathyroidism
- Hypercalcemia of malignancy

Clinical Background

Parathyroid hormone (PTH) acts to increase calcium absorption from the gut and to mobilize calcium from bone. The net effect is to increase the extracellular concentration of calcium and prevent hypocalcemia. PTH secretion by the parathyroid gland is modulated by serum calcium concentration. Low calcium stimulates and high calcium inhibits PTH secretion.

PTH levels are used to assess disorders of calcium metabolism, including primary and secondary hyperparathyroidism, tumor hypercalcemia, and hypoparathyroidism.

Method

PTH (8837X, 36736X)

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 3 pg/mL
- Analytical specificity: 100% cross-reactivity with intact PTH (amino acids 1-84) and 45% with the 7-84 C-terminal fragment; no detectable cross-reactivity with other PTH fragments or with calcitonin

***Calcium, total* (*8837X*)**

- Spectrophotometry
- Analytical sensitivity: 0.2 mg/dL

Calcium, ionized (36736X)

- Ion specific electrode
- Analytical sensitivity: 0.4 mg/dL

Specimen Requirements

***PTH and total calcium* (*8837X*)**

2 mL frozen serum

1 mL minimum (infants, 0.5 mL)

***PTH and ionized calcium* (*36736X*)**

PTH

2 mL frozen serum (no additive red top or SST red top tube); 1 mL minimum

Calcium, ionized

2 mL refrigerated serum (SST red-top tube, **centrifuged, unopened**; 0.6 mL minimum

PTH-related Protein (PTH-RP) 34478X

Clinical Use

- Differential diagnosis of hypercalcemia
- Manage patients with solid tumors and hypercalcemia

Reference Range

≤4.7 pmol/L

Interpretive Information

↑
- Tumor-associated hypercalcemia
- Fetus
- Newborn

Clinical Background

Parathyroid hormone-related protein (PTH-RP) has an amino-terminal structure similar to parathyroid hormone (PTH). PTH-RP can bind to and stimulate PTH receptors and is produced mainly by solid tumors. It is also found in the normal lactating mammary gland, keratinocytes, placenta, and parathyroid gland.

PTH-RP levels are high in approximately 70% of the patients with tumor-associated hypercalcemia. It may be useful in the differential diagnosis of hypercalcemia, specifically differentiating primary hyperparathyroidism from hypercalcemia related to cancer. It is not elevated in primary or chronic renal failure.

Method

- Immunoradiometric assay (IRMA)
- Analytical sensitivity: 2.5 pmol/mL
- Analytical specificity: intact PTH-RP; no detectable cross-reactivity with PTH (C-terminal, mid-molecule, amino acids 39-84, or intact)

Specimen Requirements

1.5 mL frozen plasma

1.0 mL minimum

Collect blood in syringe or red-top tube and immediately transfer to a cold, special Nichols Institute PTH-RP collection tube; mix thoroughly. Centrifuge in refrigerated centrifuge. Transfer plasma to a plastic tube and freeze.

Pyridinium Collagen Cross-links, Urine (PYD and DPYD) 36098X 36097X

Clinical Use

- Assess rate of bone collagen degradation
- Monitor response to therapy

Reference Range

	PYD nmol/mmol creat	*DPYD* nmol/mmol creat
Men		
24-h urine	20-61	3.7-19
2-h urine	23-65	6.2-26
Women*		
24-h urine	22-89	4.4-21
2-h urine	25-83	5.7-23
Children (24-h urine)		
2-10 y	158-442	31-112
11-14 y	107-398	17-101
15-17 y	42-200	≤59
Children (2-h urine)		
2-10 y	153-401	39-120
11-17 y	17-408	3.0-117
Tanner I	112-353	27-103
Tanner II	95-471	20-139
Tanner III	68-489	14-135
Tanner IV	17-448	10-136

Creatinine	Random urine mg/dL	24-h urine g/24-h
0-6 mo	2-32	
7-11 mo	2-36	
1-2 y	2-128	
3-8 y	2-149	0.11-0.68
9-12 y	2-183	0.17-1.41
13-17 y		0.29-1.87
Adults		0.63-2.50
>12 y		
Male	20-370	
Female	20-320	

*Premenopausal. A value within the premenopausal range does not rule out osteoporosis or the need for therapy.

Clinical Background

Collagen fibers are linked together by interchain molecules referred to as pyridinium cross-links. Collagen pyridinium cross-links have been identified in all connective tissue except skin. As collagen is broken down by collagenase, small breakdown products are excreted in urine with the attached cross-links, including pyridinoline (PYD) and deoxypyridinoline (DPYD). Bone collagen is constantly turning over and is rich in DPYD. Thus, urine PYD and, especially, DPYD are useful markers for bone matrix degradation and resorption.

Method

- High-performance liquid chromatography (HPLC)
- Analytical sensitivity: 20 pmol/mL (PYD); 13 pmol/mL (DPYD)
- Creatinine concentration also reported

Specimen Requirements

24-Hour urine (36098X)

5 mL refrigerated aliquot of a 24-h urine; 1 mL minimum

Collect urine with 25 mL 6N HCl or 10 g of boric acid. Mix and aliquot. Record total volume on vial and request form.

2-Hour urine (36097X)

5 mL refrigerated aliquot of a 2-h urine; 0.5 mL minimum

Discard first morning void. Collect urine for next 2 hours, in presence of 2 mL 6N HCl or 1 g of boric acid. Mix well and aliquot.

Interpretive Information

↑
- Osteoporosis
- Primary hyperparathyroidism
- Paget's disease

↓
- Relative to baseline indicates successful therapy

Resistance to Thyroid Hormone 16053X

Clinical Use

- Differential diagnosis of hyperthyroidism
- Screen individuals at risk for resistance to thyroid hormone

Reference Range

Negative (no mutations detected)

Interpretive Information

Mutation present

- Resistance to thyroid hormone

Clinical Background

Resistance to thyroid hormone (RTH) is caused by reduced target tissue response to thyroid hormone, resulting in the loss of the normal feedback mechanism that suppresses the secretion of thyrotropin (TSH). It is characterized by the presence of elevated serum levels of the free thyroid hormones thyroxine (T4) and triiodothyronine (T3) with slightly elevated or normal TSH. In 90% of cases, RTH is caused by mutations in the thyroid hormone receptor beta (*THRB*) gene that alter the hormone-binding region of the protein. The disorder is usually inherited in an autosomal dominant manner, and over 100 different mutations have been described. New mutations can give rise to isolated cases of RTH.

Since genetic variation and other problems can affect the accuracy of direct mutation testing, test results should always be interpreted in light of clinical and family data. Genetic counseling may be helpful for individuals and families undergoing testing for *THRB* mutations.

Method

- Polymerase chain reaction (PCR) and DNA sequencing
- Analytical specificity: mutations in exons 3-10 of the *THRB* gene and their flanking intronic regions

Specimen Requirements

5 mL room temperature whole blood

3 mL minimum

Collect in lavender-top (EDTA) tube

Serotonin

818X
29851X

Clinical Use

- Diagnose paraneoplastic carcinoid syndrome

Reference Range

	ng/mL
Blood (818X)	22-180
Serum (29851X)	
Adults	26-165
Children (1-12 y)	81-349

Pediatric reference range from *Brazilian J Med Biol Res.* 1993;26:309-317.

Interpretive Information

- ↑ Carcinoid syndrome
- ↑ Medullary thyroid carcinoma
- ↑ Multiple endocrine neoplasia (MEN) types 1 and 2
- ↑ Oat cell carcinoma of the lung
- ↑ Islet cell tumors
- ↓ Depression

Clinical Background

Carcinoid syndrome manifests with flushing, diarrhea, pain, and hepatomegaly. The ileum, stomach, pancreas, duodenum, bronchus, thymus, thyroid, testis, and ovary may be involved. Small bowel carcinoids most commonly metastasize to the liver. These tumors secrete increased amounts of serotonin, which metabolizes to 5-hydroxyindoleacetic acid (5-HIAA). Measurement of serotonin concentration may be helpful in selected cases.

Method

- High performance liquid chromatography (HPLC)
- Fluorescent detection

Specimen Requirements

Whole blood (818X)

4 mL frozen whole blood

1 mL minimum

Collect blood using the Serotonin Kit (provided by Quest Diagnostics)

Serum (29851X)

2 mL frozen serum

1 mL minimum

No additive red top preferred

SST red top acceptable

Patients should avoid avocados, bananas, tomatoes, plums, walnuts, pineapples, eggplant, tobacco, tea, and coffee for 3 d prior to specimen collection.

Sex Hormone Binding Globulin 30740X

Clinical Use

- Assess abnormal serum gonadal hormone concentrations

Reference Range

	Males	Females
Adults	nmol/L	nmol/L
18-29 y	7-49	6-112
30-39 y	8-48	14-102
40-49 y	9-45	11-100
50-59 y	18-47	17-78
60-69 y	17-54	17-95
70-79 y	23-65	21-90
80-91 y	20-63	26-77
Children		
3-9 y	18-136	18-136
10-13 y	17-123	17-123
14-17 y	11-71	11-71
Tanner stages		
I	39-155	38-114
II	33-135	24-90
III	21-72	22-112
IV	11-92	22-69
V	18-54	18-76

Interpretive Information

- Estrogen administration
- Pregnancy
- Alcoholism
- Male hypogonadism
- Hyperthyroidism
- Testicular feminization

- Hirsutism
- Virilization
- Massive obesity
- Exogenous androgen therapy or abuse

Clinical Background

The majority of circulating gonadal steroid hormones are protein bound. Testosterone, dihydrotestosterone, and the estrogens are bound to circulating sex hormone binding globulin (SHBG or testosterone-binding globulin, TeBG), which is produced by the liver. Consequently, total circulating steroid hormone levels are affected by alterations in SHBG concentration as well as concentration of the hormones themselves. Evaluation of the gonadal steroid hormone system, therefore, includes measurements of total serum testosterone, dihydrotestosterone, or estrogen species in addition to measurements of the free hormones and SHBG.

SHBG concentrations are increased by estrogens and pregnancy and decreased by testosterone. Insulin lowers SHBG levels in high insulin states (eg, diabetes, obesity, polycystic ovary syndrome).

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 2 nmol/L

Specimen Requirements

1 mL room temperature serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Specify age and sex on request form.

Somatostatin 34480X

Clinical Use

- Diagnose somatostatin-producing tumors

Reference Range

Adults	10-22 pg/mL

Interpretive Information

Increased

- Somatostatinoma
- Medullary thyroid carcinoma
- Pheochromocytoma

Decreased

- Following vagotomy

Clinical Background

Somatostatin, also referred to as somatotropin release inhibiting factor (SRIF), is a neurohormone produced as a 14-amino acid peptide in the brain and hypothalamus and as a 28-amino acid N-extended product in the gastrointestinal tract. In the pituitary gland, SRIF inhibits secretion of growth hormone, TSH, and, under certain conditions, prolactin and ACTH. It exerts inhibitory effects on all endocrine and exocrine secretions of the pancreas and gut and a variety of endocrine-secreting tumors including insulinomas, glucagonomas, VIPomas, carcinoid tumors, and gastrinomas. Pancreatic somatostatinomas cause mild diabetes mellitus, cholelithiasis, steatorrhea, indigestion, and hypochlorhydria secondary to the inhibitory effects of secreted SRIF. SRIF may also be secreted by medullary thyroid carcinomas or pheochromocytomas.

Method

- Extraction and radioimmunoassay (RIA)
- Analytical sensitivity: 10 pg/mL

Specimen Requirements

1.8 mL frozen EDTA plasma

0.6 mL minimum

Draw in pre-chilled lavender-top tube. Separate and freeze plasma immediately.

T3 Antibody 36574X

Clinical Use

- Evaluate discordant serum T3 levels

Reference Range

Negative

Interpretive Information

- Autoimmune thyroid disease
- Other autoimmune disease

Clinical Background

Patients with autoimmune thyroid diseases, including autoimmune (Hashimoto) thyroiditis and Graves disease, may rarely develop antibodies to T3 (triiodothyronine) and/or T4 (thyroxine). T3 antibodies interfere with T3 assay measurement by competing with the anti-T3 immunoglobulins used in the T3 assay.

When a patient has a serum T3 concentrati\on that is discordant with other test results, eg, elevated serum T3 in the presence of a detectable serum TSH concentration, then it may be appropriate to determine if anti-T3 antibodies are present.

Method

- Radiobinding assay (RBA)

Specimen Requirements

1 mL refrigerated serum

0.1 mL minimum

No additive red top preferred

SST red top acceptable

T3 Uptake 17732X

Clinical Use

- Assess T4 (thyroxine) protein binding
- Indirect estimate of free T4 concentration

Reference Range

Adults	0.79-1.16

Interpretive Information

↑
- Hyperthyroidism
- Renal failure
- Malnutrition

↓
- Hypothyroidism
- Pregnancy
- Acute hepatitis

Clinical Background

Thyroid hormones circulate bound to several binding proteins. The most significant among these is thyroxine-binding globulin (TBG). TBG levels are influenced by a variety of factors including genetic abnormalities, pregnancy, oral contraceptives, glucocorticoids, androgens, and renal disease. Fluctuations in TBG concentrations lead to changes in T4 and T3 levels that may generate a false diagnosis of thyroid dysfunction in basically euthyroid patients.

There are several methods of assessing TBG-binding activity in serum. The T3 uptake provides an indirect assessment of TBG binding. Chemiluminescence-labeled T3 competitively binds to a solid phase-bound antibody or the patient TBG. The T3 bound to antibody is measured relative to that in a standard serum. Increased antibody binding reflects decreased TBG binding (TBG-binding site saturation). Decreased antibody binding reflects increased TBG binding (binding site desaturation).

Method

- Immunochemiluminometric assay (ICMA)

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

T3, Free, Non-dialysis 34429X

Clinical Use

- Diagnose hyperthyroidism
- Clarify thyroid status in presence of possible protein binding abnormality

Reference Range

	pg/dL
Adults	230-420
Children	
<1 y	Not established
1-9 y	337-506
10-13 y	335-480
14-18 y	287-455

Interpretive Information

- Graves disease
- T3 thyrotoxicosis
- Thyroid hormone resistance
- Functional thyroid adenoma (T3-producing)

- Nonthyroidal illness
- Hypothyroidism (only 1/3 of cases)

Clinical Background

Most circulating T3 (triiodothyronine) is bound to plasma proteins; only 0.3% exists in the free, unbound state and is available for exchange with intracellular T3 receptors.

T3 measurements are used to diagnose and monitor treatment of hyperthyroidism (see T3, Total). When an increase in circulating thyroxine-binding proteins is suspected as the cause of an elevated total T3 level, the free T3 assay can differentiate this condition from true hyperthyroidism. Non-dialysis free T3 immunoassay methods tend to underestimate free T3 due to nonspecific absorption of free T3 to proteins or matrix reagents. Although they do provide a useful free T3 index, the most reliable free T3 measurement is the equilibrium dialysis method.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 50 pg/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

T3, Free, Tracer Dialysis 36598X

Clinical Use

- Diagnose hyperthyroidism
- Clarify thyroid status in presence of possible protein binding abnormality

Reference Range

Free T3	pg/dL
Non-pregnant adults	210-440
Pregnant women	200-380
Children	
<1 y	Not established
1-9 y	282-518
10-13 y	286-556
14-17 y	242-501

Total T3	ng/dL
Adults	60-181
Pregnancy	
1st trimester	81-190
2nd and 3rd trimester	100-260
Children	
<1 y	Not established
1-9 y	127-221
10-13 y	123-211
14-18 y	97-186

Interpretive Information

- Graves disease
- T3 thyrotoxicosis
- Thyroid hormone resistance
- Functional thyroid adenoma (T3-producing)

- Nonthyroidal illness
- Hypothyroidism (only 1/3 of cases)

Clinical Background

Most circulating T3 is bound to plasma proteins. Only 0.3% exists in the free, unbound state and is available for exchange with intracellular T3 receptors.

T3 measurements are used to diagnose and monitor treatment of hyperthyroidism (see T3, Total). When an increase in circulating thyroxine-binding protein is suspected as the cause of an elevated total T3 level, the free T3 assay can differentiate this condition from true hyperthyroidism.

Method

- Free T3: equilibrium dialysis, radioimmunoassay (RIA)
- Total T3: immunochemiluminometric assay (ICMA)
- Reported free T3 is the product of the 2: (total T3 x DFT3) where DFT3 is the dialyzable fraction of T3
- Analytical sensitivity: 0.01% and 25 ng/dL for free T3 and total T3, respectively

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

T3, Reverse 967X

Clinical Use

- Establish nonthyroidal illness as the cause of abnormal thyroid function tests

Reference Range

0.11-0.32 ng/mL

Interpretive Information

↑
- Euthyroid sick syndrome
- Fetus
- Newborn
- Low T3 syndrome
- Hyperthyroidism

↓
- Hypothyroidism

Clinical Background

3,3´5´-Triiodothyronine (reverse T3, rT3) is, along with 3,5,3´-triiodothyronine (T3), a deiodinated metabolite of thyroxine (T4), the major secretory product of the thyroid gland. Unlike T3, however, reverse T3 is metabolically inert.

The process of 5´-monodeiodination that converts T4 to T3 and rT3 to diodothyronine is inhibited in a wide variety of conditions including fasting, malnutrition, poorly controlled diabetes mellitus, trauma, surgery, and systemic illnesses. Consequently, the serum T3 level typically decreases, and the rT3 concentration often, but not always, increases in these circumstances.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 0.05 ng/mL

Specimen Requirements

1 mL refrigerated serum

0.4 mL minimum

No additive red top preferred

SST red top acceptable

T3, Total (Triiodothyronine) 859X

Clinical Use

- Diagnose hyperthyroidism
- Reflect tissue monodeiodination of T4

Reference Range

	ng/dL
Adults	60-181
Pregnancy	
1st trimester	81-190
2nd and 3rd trimester	100-260
Children	
<1 y	Not established
1-9 y	127-221
10-13 y	123-211
14-18 y	97-186

Interpretive Information

- Graves disease
- T3 thyrotoxicosis
- Hyperthyroidism, TSH dependent
- Increased TBG
- Pregnancy

- Nonthyroidal illness (low T3 syndrome)
- Hypothyroidism (1/3 of cases)
- Decreased TBG

Clinical Background

Triiodothyronine (T3) is primarily produced by extrathyroidal deiodination of T4, but 20% of it is secreted directly by the thyroid gland. Circulating T3 is predominantly bound to plasma proteins. Only 0.3% is unbound (free) and readily available for tissue exchange and interaction with its nuclear receptor.

Total T3 measurements are used to diagnose and monitor treatment of hyperthyroidism. They are essential for recognizing T3 toxicosis, more fully defining the severity of hyperthyroidism, and for detecting recurrent Graves disease. Changes in thyroxine-binding protein concentrations alter the total T3 concentration without affecting the free T3 level; thus, free T3 assays can differentiate most of these conditions from true hyperthyroidism (see T3, Free, Tracer Dialysis).

A T3 measurement is usually not helpful for diagnosing hypothyroidism, since the serum T3 concentration typically remains normal in mild and moderate thyroid gland failure. Systemic illness and malnutrition reduce extrathyroidal T3 production, causing a low total T3 concentration that should not be confused with hypothyroidism.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 25 ng/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

T4 (Thyroxine) Antibody 36576X

Clinical Use

- Evaluate discordant serum T4 levels

Reference Range

Negative

Interpretive Information

- Autoimmune thyroid disease
- Other autoimmune disease

Clinical Background

Patients with autoimmune thyroid diseases, including autoimmune (Hashimoto) thyroiditis and Graves disease, may rarely develop antibodies to T4 (thyroxine) and/or T3 (triiodothyronine). T4 antibodies interfere with T4 assay measurement by competing with the anti-T4 immunoglobulins used in the T4 assay.

When a patient has a serum T4 concentration that is discordant with other test results, eg, elevated serum T4 in the absence of thyroxine binding globulin (TBG) excess and presence of a detectable serum TSH concentration, then it may be appropriate to determine if anti-T4 antibodies are present.

Method

- Radiobinding assay (RBA)

Specimen Requirements

1 mL refrigerated serum

0.1 mL minimum

No additive red top preferred

SST red top acceptable

T4 Binding Proteins 36738X

Clinical Use

- Evaluate patients with euthyroid hyperthyroxinemia and hypothyroxinemic states

Reference Range

See next page

Interpretive Information

	Mass of T4 bound to:		
	TBG	PreALB	ALB
TBG deficiency	↓	N	N
TBG excess	↑	N	N
TBG variant	↓	N	N
TBPA excess	N	↑	N
TBPA variants	N	↑	N
Dysalbuminemic hyperthyroxinemia	N	N	↑
Euthyroid (sick) hypothyroxinemia	↓	↓	N
Hypothyroidism	↓	↓	↓
Hyperthyroidism	↑	↑	↑

	Mass of T4 bound per unit mass of:		
	TBG	PreALB	ALB
TBG deficiency	N	N	N
TBG excess	N	N	N
TBG variant	↓	N	N
TBPA excess	N	N	N
TBPA variants	N	↑	N
Dysalbuminemic hyperthyroxinemia	N	N	↑
Euthyroid (sick) hypothyroxinemia	↓	↓	N
Hypothyroidism	↓	↓	↓
Hyperthyroidism	↑	↑	↑

Clinical Background

Thyroid hormones normally circulate bound to 1 of 3 classes of plasma proteins: thyroxine-binding globulin (TBG), transthyretin (prealbumin, preALB), or albumin (see Test Application and Interpretation section). Alterations in T4-binding affinity and/or concentrations of these proteins change the total and, in some cases, the free T4 concentrations.

This thyroxine-binding protein panel reveals the precise distribution of protein-bound T4 in plasma. Thyroxine-binding globulin excess, familial dysalbuminemic hyperthyroxinemia (FDH), transthyretin-binding excess, and T4 antibodies can be differentiated. The panel can also differentiate TBG deficiency and some molecular variants of TBG.

Method

Quantify total T4 and binding proteins

- Immunochemiluminometric assay (total T4 and TBG)
- Fixed time nephelometry (prealbumin)
- Spectrophotometry (albumin)

Quantify T4 bound to each binding protein

- Electrophoresis, radiobinding assay (TBG-, prealbumin-, albumin-bound T4)

Calculate T4 affinity of each binding protein

- TBG-bound T4/TBG
- Prealbumin-bound T4/prealbumin
- Albumin-bound T4/albumin

Specimen Requirements

4 mL refrigerated serum

2 mL minimum

No additive red top preferred

SST red top acceptable

Continued

T4 Binding Proteins *(continued)* 36738X

Reference Range	
T4, Total	µg/dL
Adults	4.8-10.4
Pregnancy	
1st trimester	6.4-15.2
2nd trimester	7.4-15.2
3rd trimester	7.7-13.8
All trimesters	7.0-14.7
Children	
1-8 y	5.9-11.5
9-13 y	4.7-10.4
14-17 y	5.0-9.8
TBG-bound T4	
Adults	2.1-6.2
Children (5-17 y)	2.6-5.8
Prealbumin-bound T4	
Adults	1.0-3.3
Children (5-17 y)	1.8-4.0
Albumin-bound T4	
Adults	0.5-1.2
Children (5-17 y)	0.5-1.0
TBG	µg/mL
Men	12.7-25.1
Women	13.5-30.9
Children	
3-8 y	16.1-24.2
9-13 y	12.5-25.8
14-17 y	9.8-23.7
Prealbumin	mg/dL
Men	21-43
Women	17-34
Albumin	3700-5100
TBG-bound T4/TBG	µg/mg
Adults	1.0-3.0
Children (5-17 y)	2.0-3.0
Prealbumin-bound T4/prealbumin	
Adults	0.04-0.11
Children (5-17 y)	0.08-0.16
Albumin-bound T4/albumin	µg/g
Adults	0.11-0.28
Children (5-17 y)	0.12-0.22

T4, Free, Direct Dialysis 35167X

Clinical Use

- Differentiate euthyroid hyperthyroxinemia from hyperthyroidism
- Differentiate euthyroid hypothyroxinemia from hypothyroidism

Reference Range

	ng/dL
Adults	
21-87 y	0.8-2.7
Pregnancy	
1st trimester	0.9-2.0
2nd trimester	0.8-1.5
3rd trimester	0.8-1.7
Children (3-20 y)	1.0-2.4

Interpretive Information

↑
- Hyperthyroidism
- Thyroid hormone resistance

↓
- Primary hypothyroidism
- Secondary hypothyroidism

Clinical Background

Thyroid hormones are transported in blood bound to several binding proteins. These include thyroxine-binding globulin, prealbumin, and albumin. Only 0.03% of thyroxine (T4) is unbound or free, but this free fraction provides the hormone available to most tissues. Hyper- and hypothyroidism result from abnormal concentrations of serum free T4. Since free T4 concentrations are never high enough to influence results of total T4 immunoassays, total T4 assays measure only protein-bound T4.

The most accurate method for determining free T4 is the direct equilibrium dialysis method. Direct dialysis separates free T4 from protein-bound T4. The free T4 is then measured directly from the protein-free dialysate. Results are independent of T4-binding protein concentrations and are unaffected by the presence of molecular variants of these proteins or by circulating thyroid autoantibodies.

Method

- Direct equilibrium dialysis, radioimmunoassay (RIA)
- Analytical sensitivity: 0.2 ng/dL
- Analytical specificity: unaffected by T4 antibodies or variations in T4-binding proteins

Specimen Requirements

2 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

T4, Free, Non-dialysis 866X

Clinical Use

- Assess thyroid status in patients with abnormal total T4 concentrations
- Initial test of thyroid function in patients with suspected thyroid dysfunction (see also TSH)

Reference Range

	ng/dL
Adults	0.8-1.8
Children	
<1 y	Not established
1-9 y	0.9-1.6
10-18 y	0.9-1.4

Interpretive Information

↑
- Hyperthyroidism
- Thyroid hormone resistance

↓
- Primary hypothyroidism
- Secondary hypothyroidism

Clinical Background

Thyroid hormones are transported in blood bound to several binding proteins. These include thyroxine-binding globulin, prealbumin, and albumin. Only 0.03% of thyroxine (T4) is unbound or free, but this free fraction provides the hormone available to most tissues. Hyper- and hypothyroidism result from abnormal concentrations of serum free T4. Since serum free T4 concentrations are never high enough to influence results of total T4 immunoassays, total T4 assays measure only protein-bound T4. Determining free T4 can distinguish the euthyroid hyperthyroxinemias from hypothyroidism.

The most commonly employed free T4 methods are the 1-step and 2-step immunoassays. These methods may provide falsely low free T4 estimates owing to nonspecific absorption of free T4 to proteins or matrix reagents. Such assays do, however, provide a useful free T4 index. The most accurate method for determining free T4 is the direct equilibrium dialysis method. Results are independent of T4-binding protein concentrations and are unaffected by the presence of molecular variants of these proteins or by circulating thyroid autoantibodies.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.1 ng/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

T4, Total (Thyroxine) 17733X

Clinical Use

- Diagnose hypo- and hyperthyroidism when overt and/or due to pituitary or hypothalamic disease

Reference Range

	µg/dL
Adult	4.8-10.4
Pregnancy	
1st trimester	6.4-15.2
2nd trimester	7.4-15.2
3rd trimester	7.7-13.8
All trimesters	7.0-14.7
Pediatric	
1-8 y	5.9-11.5
9-13 y	4.7-10.4
14-17 y	5.0-9.8

Interpretive Information

↑
- Hyperthyroidism
- Increased TBG
- Familial dysalbuminemic hyperthyroxinemia
- Increased transthyretin (preALB)
- Estrogen therapy
- Pregnancy

↓
- Primary hypothyroidism
- Pituitary TSH deficiency
- Hypothalamic TRH deficiency
- Nonthyroidal illness
- Decreased TBG

Clinical Background

Thyroxine (T4, tetraiodothyronine) is the major secretory product of the thyroid gland. In blood, T4 is bound to 1 of 3 classes of proteins: thyroxine-binding globulin (TBG), transthyretin (thyroxine-binding prealbumin), or albumin. Only the free T4 fraction (0.03%) is available for exchange with tissues where it is deiodinated to triiodothyronine (T3). T3 then binds to nuclear receptors and brings about thyroid hormone actions. The serum total T4 concentration generally reflects thyroid gland secretory activity. It is elevated in most hyperthyroid patients and low in most patients with hypothyroidism.

Although the total T4 assay is analytically precise, several factors limit the test's clinical accuracy. Changes in circulating T4-binding protein concentrations alter the total T4 level without affecting the free T4 level or causing true thyroid dysfunction. Serious systemic illness causes a low total T4 in 25% of patients and a modestly elevated T4 level in 2%; consequently, the serum T4 is not an accurate measure of thyroid function in severely ill patients.

The interpretation of T4 concentrations and assessment of thyroid axis function are greatly facilitated by measurement of circulating TSH concentration.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.8 µg/dL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top tube preferred

SST red top acceptable

Tartrate Resistant Acid Phosphatase 37351X

Clinical Use

- Assess osteoclast activity
- Assess bone turnover
- Detect bone metastasis in patients with breast cancer

Reference Range

Adults	3.0-6.0 U/L

Interpretive Information

- Osteoporosis
- Osteomalacia
- Metastatic cancer
- Paget's disease
- Primary hyperparathyroidism
- Advanced renal failure

- Bisphosphonate therapy
- Anabolic steroid therapy
- Fluoride excess
- Hypoparathyroidism

Clinical Background

Tartrate resistant acid phosphatase (TRAP) is secreted primarily by osteoclasts, but also by erythrocytes and macrophages. Serum levels can be useful as a measure of bone resorption; however, the newer resorption markers (eg, N-telopeptide [NTx], free deoxypyridinoline [free DYPD, Pyrilinks® D], and C-telopeptide [CTx, CrossLaps®]) are more reliable. TRAP is a potential marker of bone metastasis in patients with breast cancer; levels appear to increase in proportion to the number of bone metastases.

TRAP is adversely affected by enzyme inhibitors in serum, has limited stability and analytical sensitivity, and lacks specificity for bone. Erythrocytic acid phosphatase released during in vitro clotting, as well as other non-bone sources, may falsely increase values.

Method

- Enzymatic
- Analytical sensitivity: 1.2 U/L
- Analytical specificity: measures level of acid phosphatase that is resistant to tartrate inhibition

Specimen Requirements

1.0 mL frozen serum

0.3 mL minimum

No additive red top preferred

SST red top acceptable

Refrigerate prior to serum separation; freeze immediately afterwards.

12-h fast required.

TBG (Thyroxine-Binding Globulin) 870X

Clinical Use

- Differentiate quantitative TBG derangements from thyroid dysfunction

Reference Range

	µg/mL
Men	12.7-25.1
Women	13.5-30.9
Children	
3-8 y	16.1-24.2
9-13 y	12.5-25.8
14-17 y	9.8-23.7

Interpretive Information

- Pregnancy
- Infancy
- Familial excess
- Hepatitis
- Estrogen treatment
- Tamoxifen treatment

- Androgenic steroid treatment
- Glucocorticoids (large dose)
- Nephrotic syndrome
- Acromegaly
- Familial deficiency

Clinical Background

Thyroxine-binding globulin (TBG) is a glycoprotein produced in the liver. TBG binds both thyroxine (T4) and triiodothyronine (T3) with high affinity and accounts for 75% of plasma protein thyroxine-binding activity. Thus, an increase or decrease in its circulating level alters total concentrations of T4 and T3 in blood, leading to potential confusion with true thyroid gland function. A number of diseases and medications, as well as inherited alterations in TBG gene expression, can change the serum TBG concentration (see Test Application and Interpretation section). Common causes of increased serum TBG include estrogen therapy (especially oral contraceptive agents), pregnancy, and hepatitis. Conversely, serum TBG may be decreased in cirrhosis, in the nephrotic syndrome, and by androgens.

The measurement of TBG can be used to establish the presence of TBG deficiency or excess suggested by abnormal total serum T4 and T3 concentrations in the presence of normal free levels of these hormones. Definitive documentation of a TBG derangement may avoid unnecessary diagnostic procedures and therapy in individuals with harmless congenital TBG anomalies, and in their relatives.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 3.5 µg/mL

Specimen Requirements

1 mL refrigerated serum (no additive red top tube); 0.5 mL minimum

SST red top unacceptable

TBII (Thyrotropin-binding Inhibitory Immunoglobulin) 5738X

Clinical Use

- Diagnose and manage Graves disease, neonatal hypothyroidism, and postpartum thyroid dysfunction

Reference Range

Adults and children: ≤16% inhibition

Graves disease: 16% - 100% inhibition

Interpretive Information

- Graves disease
- Atrophic thyroiditis
- Postpartum autoimmune thyroid disease
- Neonatal Graves disease
- Transient neonatal hypothyroidism

Clinical Background

Autoimmune thyroid diseases are associated with thyroid autoantibodies, including several types directed against the TSH receptor. TSH receptor autoantibodies may be stimulatory, mimicking the actions of TSH, as in Graves disease. Alternatively, they may inhibit TSH binding and block the action of endogenous TSH. Such blocking autoantibodies produce transient neonatal hypothyroidism and chronic atrophic thyroiditis. TBII, which measures the ability of antibodies to inhibit TSH binding to its receptor, reflects the presence of either or both the stimulatory and inhibitory immunoglobulin classes.

Method

- Radioreceptor assay
- Analytical sensitivity: 2% inhibition

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Testosterone, Calculated Free, Bioavailable, and Total 14966X

Clinical Use

- Assess androgen status in male hypogonadism; female hirsutism, virilization, acne, and amenorrhea; or in pediatric patients

Reference Range

See next page.

Interpretive Information

- Polycystic ovarian disease
- Cushing's disease
- Congenital adrenal hyperplasia
- Androgen resistance

- Hypogonadism
- P-450$_{c17}$ enzyme deficiency
- Delayed puberty in boys
- Gonadotropin deficiency
- Testicular defects

Clinical Background

Most testosterone is transported in blood by sex hormone binding globulin (SHBG). Free testosterone is the small amount of testosterone circulating unbound. Blood testosterone levels are dependent on rates of production, interconversion, metabolic clearance, and SHBG concentration. SHBG levels are altered by medications, disease, sex steroids, and insulin. SHBG increases with age in men and decreases with androgen excess in hirsute women. Thus, determination of free testosterone more accurately reflects the level of bioactive testosterone than measurements of total testosterone and offers greater sensitivity in diagnosis of hypogonadism in aging men and evaluation of mildly hyperandrogenemic women.

In general, total testosterone by ICMA, a less technically demanding and more economical procedure, is satisfactory for screening potential male hypogonadism. The Endocrine Society has stated that ICMA or other "direct" assays are unreliable for diagnosing patients with low levels (eg, <300 ng/dL) of testosterone.

Method

Testosterone, Total

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)

Testosterone, Free

- Calculation

Testosterone, Bioavailable

- Calculation

SHBG

- Immunochemiluminometric assay (ICMA)

Albumin

- Spectrophotometry

Specimen Requirements

2.8 mL refrigerated serum (no additive red top tube); 1.3 mL minimum

SST red top unacceptable

Continued

Testosterone, Calculated Free, Bioavailable, and Total *(continued)* 14966X

Reference Range

Testosterone, Free	Males	Females
Adults	pg/mL	pg/mL
18-69 y	46.0-224.0	0.2-5.0
70-89 y	6.0-73.0	0.3-5.0
Children		
1-10.9 y	≤1.3	≤1.5
11-11.9 y	≤1.3	≤1.5
12-13.9 y	≤64.0	≤1.5
14-17.9 y	4.0-100.0	≤3.6
Testosterone, Bioavailable	ng/dL	ng/dL
Adults		
18-69 y	110.0-575.0	0.5-8.5
70-89 y	15.0-150.0	0.5-8.8
Children		
1-11.9 y	≤5.4	≤3.4
12-13.9 y	≤140.0	≤3.4
14-17.9 y	8.0-210.0	≤7.8
SHBG	nmol/L	nmol/L
Adults		
18-29 y	7-44	8-112
30-39 y	5-49	9-107
40-49 y	8-46	8-96
50-59 y	15-49	10-89
60-69 y	23-38	20-102
70-79 y	21-68	21-76
80-91 y	20-63	
80-84 y		26-77
Children		
3-9 y	18-136	18-136
10-13 y	17-123	17-123
14-17 y	11-71	11-71
Tanner stages		
I	39-155	38-114
II	33-135	24-90
III	21-72	22-112
IV	11-92	22-69
V	18-54	18-76
Albumin	3.6-5.1 g/dL	

See ***Testosterone, Total (Women, Children, Hypogonadal Males), LC/MS/MS*** for total testosterone reference ranges.

Testosterone, Free and Total, LC/MS/MS 36170X

Clinical Use

- Assess androgen status in male hypogonadism; female hirsutism, virilization, acne, and amenorrhea; or in pediatric patients

Reference Range

	Free	*Free*
Men	%	pg/mL
18-69 y	1.5-2.2	35.0-155.0
70-89 y	1.5-2.2	30.0-135.0
Women		
18-69 y	0.5-2.0	0.1-6.4
70-89 y	0.5-2.0	0.2-3.7
Pregnancy		
1st trimester	0.15-0.66	0.5-6.0
2nd trimester	0.10-0.34	0.2-3.1
3rd trimester	0.15-0.51	0.2-4.1
Children, males		
5-9 y	0.44-1.78	≤5.3
10-13 y	0.53-3.33	0.7-52.0
14-17 y	1.05-2.91	18.0-111.0
Children, females		
5-9 y	0.28-1.81	0.2-5.0
10-13 y	0.36-3.16	0.1-7.4
14-17 y	0.41-2.34	0.5-3.9

See ***Testosterone, Total (Women, Children, Hypogonadal Males), LC/MS/MS*** for total testosterone reference ranges.

Interpretive Information

- Polycystic ovarian disease
- Hyperthecosis
- Cushing's disease
- Congenital adrenal hyperplasia
- Precocious puberty
- Androgen resistance

- Hypogonadism
- P-450$_{c17}$ enzyme deficiency
- Delayed puberty in boys
- Gonadotropin deficiency
- Testicular defects

Clinical Background

Most testosterone is transported in blood by sex hormone binding globulin (SHBG). Free testosterone is the small amount of testosterone circulating unbound. Blood testosterone levels are dependent on rates of production, interconversion, metabolic clearance, and SHBG concentration. SHBG levels are altered by medications, disease, sex steroids, and insulin. SHBG increases with age in men and decreases with androgen excess in hirsute women. Thus, determination of free testosterone more accurately reflects the level of bioactive testosterone than measurements of total testosterone and offers greater sensitivity in diagnosis of hypogonadism in aging men and evaluation of mildly hyperandrogenemic women.

In general, total testosterone by ICMA, a less technically demanding and more economical procedure, is satisfactory for screening potential male hypogonadism. The Endocrine Society has stated that ICMA or other "direct" assays are unreliable for diagnosing patients with low levels (eg, <300 ng/dL) of testosterone.

Method

Total Testosterone

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 ng/dL

Free Testosterone

- Tracer equilibrium dialysis, calculation

Specimen Requirements

0.9 mL refrigerated serum (no additive red top tube); 0.38 mL minimum

SST red top unacceptable

Testosterone, Total, ICMA 873X

Clinical Use

- Assess testicular function in males

The preferred test for women, children, and hypogonadal men is ***Testosterone, Total (Women, Children, Hypogonadal Males), LC/MS/MS (test code 15983X)***.

Reference Range

Males (20-60 y)	241-827 ng/dL

Interpretive Information

↑
- Androgen resistance

↓
- Delayed puberty (males)
- Gonadotropin deficiency
- Testicular defects
- Systemic diseases

Clinical Background

Testosterone is secreted by the testes in the male and by both the adrenal and the ovary in the female. It is the most potent of the circulating androgenic hormones and perhaps the most reliable for clinical assessment of androgenic effects. Circulating testosterone is largely bound to sex hormone binding globulin (SHBG) and to albumin; only 2% is free. Testosterone levels are decreased in primary (increased LH) and secondary (decreased LH) hypogonadism in the male, as well as in delayed puberty in boys.

This test is best utilized as a screening test: if results are ≥300 ng/dL, there is very little chance of hypogonadism. Aging men with testosterone levels frequently just below or close to the lower limit of the reference range may not be recognized as hypogonadal by this assay. The Endocrine Society has stated that ICMA or other "direct" assays are unreliable for diagnosing patients with low levels of testosterone. For such cases, the LC/MS/MS method should be used (test code 15983X).

Aging men with clinically significant hypogonadal symptoms and testosterone levels repeatedly ≤200-300 ng/dL may benefit from testosterone treatment after adequate risk/benefit counseling.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 ng/dL

Specimen Requirements

2 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Specify age and sex on test request form.

Testosterone, Total (Women, Children, Hypogonadal Males) 15983X

Clinical Use

- Assess androgen status in male hypogonadism, in children, and in female hirsutism, virilization, acne, and amenorrhea

Reference Range

	ng/dL	ng/dL
Adults	Men	Women
18-69 y	250-1100	2-45
70-89 y	90-890	
70-94 y		2-40
1st trimester pregnancy		20-135
2nd trimester pregnancy		11-153
3rd trimester pregnancy		11-146

Children	Males	Females
Cord blood*	17-61	16-44
1-10 d*	≤187	≤24
1-3 mo*	72-344	≤17
3-5 mo*	≤201	≤12
5-7 mo*	≤59	≤13
7-12 mo*	≤16	≤11
1-5.9 y	≤5	≤8
6-7.9 y	≤25	≤20
8-10.9 y	≤42	≤35
11-11.9 y	≤260	≤40
12-13.9 y	≤420	≤40
14-17.9 y	≤1000	≤40
Tanner stages		
I	≤5	≤8
II	≤167	≤24
III	21-719	≤28
IV	25-912	≤31
V	110-975	≤33

*Data from *J Clin Invest.* 1974;53:819-828 and *J Clin Endocrinol Metab.* 1973;36:1132-1142.

Interpretive Information

- Polycystic ovarian disease
- Precocious puberty
- Cushing's syndrome
- Congenital adrenal hyperplasia
- Androgen resistance

- Hypogonadism
- $P450_{c17}$ enzyme deficiency
- Delayed puberty in boys
- Gonadotropin deficiency
- Testicular defects

Clinical Background

Testosterone is secreted by the testes in the male and by both the adrenal and the ovary in the female. It is the most potent of the circulating androgenic hormones and perhaps the most reliable for clinical assessment of androgenic effects. Circulating testosterone is largely bound to sex hormone binding globulin (SHBG) and to albumin; only 2% is free. Testosterone levels are decreased in primary (increased LH) and secondary (decreased LH) hypogonadism in the male, as well as in delayed puberty in boys. Testosterone concentrations may be increased in hirsutism and virilization in the female.

The Endocrine Society recommends use of a highly sensitive method (eg, LC/MS/MS, but not ICMA or other "direct" assay) whenever low testosterone levels are suspected (eg, women, children, and hypogonadal males). This includes aging men with clinically significant hypogonadal symptoms and testosterone levels repeatedly ≤200-300 ng/dL; these men may benefit from testosterone treatment after adequate risk/benefit counseling.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 1 ng/dL

Specimen Requirements

0.5 mL refrigerated serum (no additive red top tube); 0.18 mL minimum

SST red top unacceptable

Testosterone, Urine 874X

Clinical Use

- Assess androgen status as cumulative urinary excretion over 24-h period

Reference Range

Testosterone	µg/24-h
Men	20-170
Women	5-38
	µg/g creat
Men	13-110
Women	3-47
Creatinine	g/24-h
3-8	0.11-0.68
9-12	0.17-1.41
13-17	0.29-1.87
Adults	0.63-2.50

Interpretive Information

↑
- Androgen resistance
- Cushing's syndrome
- Polycystic ovarian disease
- Congenital adrenal hyperplasia

↓
- Hypogonadism
- Gonadotropin deficiency
- Testicular defects
- $P450_{c17}$ enzyme deficiency
- Delayed puberty in boys

Clinical Background

Testosterone is secreted by the testes in the male and by both the adrenal gland and ovaries in the female. It is the most potent of the circulating androgenic hormones and perhaps the most reliable for clinical assessment of androgenic effects. Circulating testosterone is largely bound to sex hormone binding globulin (SHBG) and to albumin; only 2% is free. Testosterone is metabolized to inactive metabolites in a variety of body tissues including muscle and adipose tissue. Inactivation is largely via hepatic cytochrome P450-3A family enzymes followed by conjugation to glucuronides for renal excretion. Twenty-four hour renal excretion is a reasonable surrogate for production rate.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Analytical sensitivity: 1 µg/L

Specimen Requirements

5 mL frozen aliquot of a 24-h urine

2.1 mL minimum

Refrigerate during collection; do not use preservatives. Record 24-h volume on vial and request form.

Tetrahydroaldosterone, 24-Hour Urine

39613X

Clinical Use

- Assess aldosterone production
- Diagnose hypo- and hyperaldosteronism

Reference Range

Tetrahydroaldosterone	
Adults	6-79 µg/24-h 6-50 µg/g creat

Creatinine	g/24-h
3-8	0.11-.68
9-12	0.17-1.41
13-17	0.29-1.87
≥18	0.63-2.50

Interpretive Information

- Primary hyperaldosteronism
- Secondary hyperaldosteronism

- Hypoaldosteronism
- Liddle's syndrome (pseudoaldosteronism)
- Apparent mineralocorticoid excess (AME)
- Licorice root or tobacco chewing

Clinical Background

Tetrahydroaldosterone is a urinary metabolite of aldosterone and serves as an index of aldosterone secretion. Measurement is useful in the diagnosis of disorders of aldosterone production, since it provides an integrated measure of the 24-hour plasma aldosterone level.

Method

- Gas chromatography/mass spectrometry (GC/MS)
- Analytical sensitivity: 1 µg/L

Specimen Requirements

5.0 mL frozen aliquot of a 24-h urine collection; 2.1 mL minimum

Refrigerate during collection; do not use preservatives.

Thyroglobulin Antibody 267X

Clinical Use

- Confirm presence of autoimmune thyroid disease

Reference Range

<20 IU/mL

Interpretive Information

- Hashimoto thyroiditis (HT)
- Primary hypothyroidism (due to HT)
- Graves disease
- Postpartum thyroiditis

Clinical Background

Circulating immunoglobulins directed against thyroglobulin are commonly present in patients with autoimmune (Hashimoto) thyroiditis and, to a lesser extent, Graves disease. Thyroglobulin is the 660-kd protein precursor of thyroid hormones. Thyroglobulin antibodies are present in the sera of approximately 70% of patients with autoimmune thyroiditis.

The principal use of this test is to confirm that a patient's diffuse goiter and/or hypothyroidism is due to autoimmune thyroiditis. The test may also help distinguish Graves disease from toxic nodular goiter. Although thyroglobulin antibodies are present less often than thyroid microsomal antibodies (eg, thyroid peroxidase antibodies, anti-TPO) in patients with autoimmune thyroid diseases, screening for both immunoglobulins increases diagnostic sensitivity.

Thyroglobulin antibodies may be detected in individuals without clinically significant thyroid disease. They do not define the patient's thyroid functional status.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 IU/mL

Specimen Requirements

1 mL room temperature serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Thyroglobulin Panel 30278X

Clinical Use

- Screen patients with thyroid carcinoma for metastases after thyroid gland ablation
- Indicate presence of thyroid gland tissue in infants with congenital hypothyroidism
- Indicate deficient thyroglobulin synthesis in infants with goitrous hypothyroidism

Reference Range

Thyroglobulin	ng/mL
Adults	2.0-35.0
Thyroglobulin Antibody	IU/mL
Adults and children	<20*

*Higher levels can interfere with thyroglobulin quantification.

Interpretive Information

Increased
- Graves disease
- Multinodular goiter
- Papillary and follicular thyroid carcinomas
- Thyroiditis
- TSH-dependent hyperthyroidism

Decreased
- Thyroidectomy
- Thyroid aplasia
- Thyroglobulin synthetic defect
- Exogenous thyroid hormone use

Clinical Background

Thyroglobulin (Tg) is a 660-kd protein synthesized exclusively in the thyroid gland. It serves as the precursor molecule for thyroid hormones, since it contains tyrosine moieties that are iodinated to form iodotyrosines and coupled to form the thyroid hormones T4 and T3. Most synthesized Tg is stored as colloid for later pinocytosis and hydrolysis within thyroid follicular cells. Small amounts are secreted directly from the endoplasmic reticulum during synthesis. Tg release is increased in a variety of thyroid disease states.

Tg measurements are most useful in the postoperative monitoring of patients with papillary, follicular, and Hürthle cell carcinomas. Tg measurement also is helpful in the differential diagnosis of congenital hypothyroidism and in detecting thyrotoxicosis caused by exogenous medication (iatrogenic or factitious), in which case the serum Tg concentration should be low. Measurements are always preceded by assessment of Tg antibodies that interfere in the assay. In the presence of endogenous antibody, the Tg measurement is not reliable.

Method

Thyroglobulin
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.2 ng/mL
- Analytical specificity: underestimation caused by thyroglobulin autoantibodies

Thyroglobulin Antibody
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 IU/mL

Specimen Requirements

2 mL room temperature serum
0.8 mL minimum

No additive red top preferred
SST red top acceptable

Thyroglobulin Panel with HAMA Treatment 19584X

Clinical Use

- Screen patients with thyroid carcinoma for metastases or recurrence

Reference Range

Thyroglobulin	ng/mL
Adults	2.0-35.0

Thyroglobulin Antibody	IU/mL
Adults and children	<20*

*Higher levels can interfere with thyroglobulin quantification.

Interpretive Information

↑
- Graves disease
- Multinodular goiter
- Papillary and follicular thyroid carcinomas
- Thyroiditis
- TSH-dependent hyperthyroidism

↓
- Thyroidectomy
- Thyroid aplasia
- Thyroglobulin synthetic defect
- Exogenous thyroid hormone use

Clinical Background

Heterophilic antibodies, defined as antibodies against animal immunoglobulins, can be a source of interference in many immunoassays. The most frequently characterized interference comes from endogenous human anti-mouse antibodies (HAMA), which can react with the mouse monoclonal antibodies utilized in the assay. HAMA presence in the patient sample can lead to over- or underestimation of thyroglobulin. In this test, HAMA is removed by precipitation prior to thyroglobulin or thyroglobulin antibody determination.

Method

- HAMA precipitation

Thyroglobulin
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.2 ng/mL
- Analytical specificity: underestimation caused by thyroglobulin autoantibodies

Thyroglobulin Antibody
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 IU/mL

Specimen Requirements

2 mL room temperature serum

1.5 mL minimum

No additive red top preferred

SST red top acceptable

Thyroid Peroxidase and Thyroglobulin Antibodies

7260X

Clinical Use

- Confirm presence of autoimmune thyroid disease

Reference Range

	IU/mL
Thyroid Peroxidase Antibody	<35
Thyroglobulin Antibody	<20

Interpretive Information

- Hashimoto thyroiditis
- Primary hypothyroidism
- Graves disease
- Postpartum thyroiditis

Clinical Background

Thyroid peroxidase (TPO) antibodies are present in approximately 95% of patients with Hashimoto thyroiditis and 85% of patients with Graves disease. Thyroglobulin (Tg) antibodies are positive in about 60% and 30% of adult Hashimoto and Graves disease patients, respectively. Tg antibodies are less frequently detected in children with these diseases. For additional information, see Clinical Background for the individual tests (267X and 5081X).

Method

Thyroid Peroxidase Antibody

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 10 IU/mL

Thyroglobulin Antibody

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 20 IU/mL

Specimen Requirements

1 mL refrigerated serum

0.6 mL minimum

No additive red top preferred

SST red top acceptable

Thyroid Peroxidase Antibody (Anti-TPO) 5081X

Clinical Use

- Confirm presence of autoimmune thyroid disease

Reference Range

<35 IU/mL

Interpretive Information

- Hashimoto thyroiditis (HT)
- Primary hypothyroidism (due to HT)
- Graves disease
- Postpartum thyroiditis

Clinical Background

Thyroid peroxidase (TPO) is an enzyme that catalyzes the iodination and coupling steps in thyroid hormone biosynthesis. It is now known to be the principal microsomal antigen against which antibodies are directed in autoimmune thyroid diseases. The main use of this test is to confirm that a patient's diffuse goiter and/or hypothyroidism is due to autoimmune thyroiditis. The test may also help distinguish Graves disease from toxic nodular goiter. The TPO antibody measurement has replaced the thyroid antimicrosomal antibody (AMA) measurement.

TPO antibodies may be detected in individuals without clinically significant thyroid disease. They do not define the patient's thyroid functional status.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 10 IU/mL

Specimen Requirements

1 mL refrigerated serum

0.5 mL minimum

No additive red top preferred

SST red top acceptable

Thyrotropin-releasing Hormone (TRH) 36588X

Clinical Use

- Potential marker for TRH-secreting tumors

Reference Range

<5 pg/mL

Interpretive Information

- After TRH injection
- Fetus and newborn
- Ectopic (extrahypothalamic) production
- Primary hypothyroidism (variable)

Clinical Background

Thyrotropin-releasing hormone (TRH) is a tripeptide produced in the hypothalamus, other central nervous system sites, and the gastrointestinal tract, especially the pancreas. TRH stimulates TSH synthesis and release. Most circulating TRH, which is rapidly degraded, is probably derived from extra-hypothalamic tissues. Circulating TRH levels are increased in fetal and neonatal serum because of increased TRH production from fetal gut tissues and placenta and low levels of TRH-degrading activity in fetal blood. Plasma TRH levels have not been shown to be useful in diagnosis of any known thyroid condition.

Method

- Radioimmunoassay (RIA)
- Analytical sensitivity: 5 pg/mL

Specimen Requirements

2 mL frozen plasma

0.6 mL minimum

Collect blood in syringe or red-top tube and immediately transfer to a cold, special Nichols Institute PTH-RP collection tube; mix thoroughly. Centrifuge in refrigerated centrifuge. Transfer plasma to a plastic tube and freeze.

TSH Antibody 36577X

Clinical Use

- Evaluate discordant serum TSH levels

Reference Range

Negative

Interpretive Information

- Autoimmune thyroid disease
- Other autoimmune disease

Clinical Background

Patients may rarely develop antibodies to thyroid stimulating hormone (TSH). These TSH antibodies interfere with the TSH assay measurement by competing with the anti-TSH immunoglobins used in the assay.

When a patient has a serum TSH concentration that is discordant with other test results, it may be appropriate to determine if TSH antibodies are present. For example, a TSH antibody may be the cause of an inappropriately reduced serum TSH level in a patient with simultaneously reduced free thyroid hormone levels (free T4 and/or free T3). Other causes of inappropriate TSH levels must also be considered:

- Hypopituitarism
- Isolated TSH deficiency
- Hypothalamic disease
- Nonthyroidal illness
- Subclinical thyrotoxicosis
- Normal elderly

Method

- Radiobinding assay (RBA)

Specimen Requirements

1 mL refrigerated serum

0.1 mL minimum

No additive red top preferred

SST red top acceptable

TSH, Ultrasensitive 8660X

Clinical Use

- Diagnose hypo- and hyperthyroidism
- Monitor T4-replacement or T4-suppressive therapy
- Quantify TSH levels in the sub-normal range

Reference Range

Adults	mU/L
Men and non-pregnant women	0.40-4.50
Pregnant women	
1st trimester	0.30-4.50
2nd trimester	0.50-4.60
3rd trimester	0.80-5.20
Children	
1-4 d	3.20-35.00
5-6 d	Not established*
1-4 wk	1.70-9.10
1-12 mo	0.80-8.20
1-19 y	0.50-4.30

*TSH levels decline rapidly during the first week of life in most children, but may remain transiently elevated in a few individuals despite normal free T4 levels. For confirmatory testing following a newborn thyroid screen, a free (or total) T4 level is usually required for proper interpretation of TSH levels in this age group.

Interpretive Information

- Primary hypothyroidism
- Decreased thyroid reserve (sub-clinical hypothyroidism)
- TSH-dependent hyper-thyroidism
- Thyroid hormone resistance

- Graves disease
- Autonomous thyroid hormone secretion
- TSH deficiency

Clinical Background

The serum TSH measurement is 1 of the most important tools in the diagnosis of thyroid disorders. TSH secretion from the pituitary gland is controlled by hypothalamic TRH and a negative feedback effect from circulating, free thyroid hormones. Thus, in subjects with a normal hypothalamic-pituitary system, there is an inverse correlation between free thyroid hormone and TSH concentrations in serum. Increased serum TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. A third-generation TSH assay using chemiluminescent technology produces functional sensitivities of 0.01 mU/L, permitting reliable quantification of serum TSH concentrations in the subnormal range. This allows differentiation of euthyroid from hyperthyroid patients. This assay also helps diagnose essentially all patients with TSH-independent hyperthyroidism (Graves disease, etc.) with a single blood sample. In addition, the assay allows adjustment of exogenous thyroxine dosage in hypothyroid patients and in patients on suppressive thyroxine therapy for thyroid neoplasia.

Method

- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.01 mU/L

Specimen Requirements

2 mL refrigerated serum (no additive red top tube); 0.5 mL minimum

TSH with HAMA Treatment 19537X

Clinical Use

- Monitor T4-replacement or T4-suppressive therapy

Reference Range

TSH, Ultrasensitive	mU/L
Men and non-pregnant women	0.40-4.50
Pregnant women	
1st trimester	0.30-4.50
2nd trimester	0.50-4.60
3rd trimester	0.80-5.20
Children	
1-4 d	3.20-35.00
5-6 d	Not established*
1-4 wk	1.70-9.10
1-12 mo	0.80-8.20
1-19 y	0.50-4.30

*TSH levels decline rapidly during the first week of life in most children, but may remain transiently elevated in a few individuals despite normal free T4 levels. For confirmatory testing following a newborn thyroid screen, a free (or total) T4 level is usually required for proper interpretation of TSH levels in this age group.

Interpretive Information

- Primary hypothyroidism
- Decreased thyroid reserve (sub-clinical hypothyroidism)
- TSH-dependent hyperthyroidism
- Thyroid hormone resistance

- Graves disease
- Autonomous thyroid hormone secretion
- TSH deficiency

Clinical Background

Heterophilic antibodies, defined as antibodies against animal immunoglobulins, can be a source of interference in many immunoassays. The most frequently characterized interference comes from endogenous human anti-mouse antibodies (HAMA), which can react with the mouse monoclonal antibodies utilized in the assay. HAMA presence in the patient sample can lead to over- or underestimation of thyroid stimulating hormone (TSH). In this test, HAMA is removed by precipitation prior to TSH determination.

Method

- HAMA precipitation
- Immunochemiluminometric assay (ICMA)
- Analytical sensitivity: 0.01 mU/L

Specimen Requirements

2 mL refrigerated serum

1.5 mL minimum

No additive red top preferred

SST red top acceptable

TSI (Thyroid Stimulating Immunoglobulin) 30551X

Clinical Use

- Diagnose and manage Graves disease

Reference Range

≤125% of basal activity

Interpretive Information

- Graves disease
- Hashitoxicosis
- Neonatal thyrotoxicosis

Clinical Background

Autoimmune thyroid disease is associated with a variety of thyroid-related autoantibodies. These include anti-thyroglobulin, anti-thyroid peroxidase, and TSH receptor antibodies. The TSH receptor antibodies may be stimulatory, exerting a TSH-like effect, or inhibitory, blocking the effect of TSH. In the thyroid follicular cell, TSH stimulates cyclic AMP (cAMP), which serves a pivotal role in thyroid cellular activation. Because TSI also stimulates cAMP production, the measurement of TSI bioactivity is accomplished by measuring its capacity to increase production of cAMP in a TSH receptor-expressing cell line.

Studies have shown that TSI is present in 80% to 90% of patients with active Graves disease and absent in some patients with remission. In patients with Graves disease, monitoring TSI levels during pregnancy helps predict neonatal Graves disease. Patients with preexisting Hashimoto thyroiditis who develop hyperthyroidism will also often manifest measurable TSI bioactivity.

TSH receptor antibodies can also be measured by their capacity to inhibit TSH binding to TSH receptors. In this case, they are referred to as TSH receptor binding inhibiting immunoglobulins (TBII, test code 5738X).

Method

- In vitro bioassay (luciferase)
- Analytical sensitivity: 75%
- Analytical specificity: underestimation caused by thyrotropin blocking antibody presence

Specimen Requirements

1 mL refrigerated serum

0.2 mL minimum

No additive red top preferred

SST red top acceptable

Vasoactive Intestinal Polypeptide (VIP) 920X

Clinical Use

- Diagnose watery diarrhea syndrome (WDS)
- Assess patients with suspected multiple endocrine neoplasia (MEN)

Reference Range

Adults and children	<50 pg/mL

Interpretive Information

- VIPoma/watery diarrhea syndrome
- MEN type 1

Clinical Background

Vasoactive intestinal polypeptide (VIP) is a 28-amino acid neuropeptide produced by neural stimulation in a variety of tissues. It has broad biological effects on the cardiovascular, gastrointestinal, and respiratory systems. These effects include increased secretion of bicarbonate and electrolytes and decreased absorption of electrolytes and water.

VIPomas can occur as part of the type 1 multiple endocrine neoplasia (MEN) syndrome. VIP is the principal mediator of the watery diarrhea syndrome (also known as pancreatic cholera, Werner-Morrison syndrome, or diarrheogenic islet cell tumor). Watery diarrhea syndrome, with elevated VIP, has also been associated with neurogenic tumors (ganglioneuroma, neuroblastoma, pheochromocytoma), bronchogenic tumors, and islet cell hyperplasia.

Method

- Extraction, radioimmunoassay (RIA)
- Analytical sensitivity: 12.5 pg/mL

Specimen Requirements

3 mL frozen EDTA plasma

1.1 mL minimum

Vitamin D, 1,25-Dihydroxy 4729X

Clinical Use

- Measurement of bioactive vitamin D
- Differential diagnosis of hypocalcemia
- Monitor patients with renal osteodystrophy or chronic renal failure

Reference Range

	pg/mL
Adults	15-60
Children	
3-17 y	27-71

Interpretive Information

↑
- Chronic granulomatous disorders
- Hyperparathyroidism
- Hypercalcemia associated with lymphoma
- Type II vitamin D-dependent rickets

↓
- Severe vitamin D deficiency
- Type I vitamin D-resistant rickets
- Hypoparathyroidism
- Pseudohypoparathyroidism
- Renal osteodystrophy, renal failure
- Tumor-induced osteomalacia
- Tumor-induced hypercalcemia (except with lymphoma)

Clinical Background

Vitamin D is a fat-soluble vitamin that is synthesized from cholesterol when the skin is exposed to ultraviolet light; it can also be derived from the diet. The liver hydroxylates vitamin D to 25-hydroxyvitamin D [25(OH)D], the major circulating species of vitamin D. The kidney, along with placenta and granuloma tissues, hydroxylates 25(OH)D to form 1, 25-dihydroxyvitamin D [1,25$(OH)_2$D], the bioactive form of the vitamin. Kidney 1-hydroxylation of 25(OH)D is stimulated by parathyroid hormone (PTH) and hypophosphatemia. Levels of 1,25$(OH)_2$D are approximately 1,000- fold less than those of 25(OH)D.

1,25$(OH)_2$D increases intestinal absorption of calcium and is required to maintain adequate bone calcification. Vitamin D deficiency is assessed with measurements of 25(OH)D. Measurements of 1,25$(OH)_2$D assess bioactive vitamin and may be useful in renal disease, parathyroid disease, and vitamin D resistance or dependence.

Method

- Extraction, chromatography, radioreceptor assay
- Analytical sensitivity: 5 pg/mL

Specimen Requirements

3 mL frozen serum

1.1 mL minimum

No additive red top preferred

SST red top acceptable

Vitamin D, 25-Hydroxy, LC/MS/MS 17306X

Clinical Use

- Diagnose vitamin D deficiency or intoxication

Reference Range

Total 25OHD	20-100 ng/mL
$25OHD_2$	Not established
$25OHD_3$	Not established

25OHD levels <20 ng/mL reflect vitamin D deficiency. The optimal level is 30-50 ng/mL. $25OHD_2$ levels >4 ng/mL suggest compliance with supplements.

Interpretive Information

↑
- Vitamin D intoxication

↓
- Nutritional rickets
- Secondary hyperparathyroidism
- Osteomalacia
- Severe cholestatic or parenchymal liver disease
- Vitamin D-resistant metabolic bone disease due to multiple drugs for seizure control
- Nephrotic syndrome with marked proteinuria
- Intestinal malabsorption

Decreased, but usually not below reference range

- Obesity
- Sarcoidosis
- Hyperphosphatemic tumoral calcinosis
- Tuberculosis
- Primary hyperparathyroidism
- Type II vitamin D-dependent rickets

Levels vary with exposure to sunlight, peaking in the summer months.

Clinical Background

25-Hydroxyvitamin D (25OHD) is the major circulating form of vitamin D and the precursor of the active form (1,25-dihydroxyvitamin D). Because of its long half-life, 25OHD measurements are useful for assessing vitamin D status in patients.

Vitamin D occurs in 2 forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is obtained from foods of animal origin and from ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin, whereas vitamin D_2 is obtained from foods of plant origin. Vitamin D_2 is used in a high potency (50,000 IU) formulation for treating severe vitamin D deficiency. Both forms are used in over-the-counter supplements and fortified foods and are metabolized to their respective 25OHD forms (ie, $25OHD_3$ and $25OHD_2$). Thus, analytical methods that can accurately quantitate both forms are essential for diagnosis and monitoring patients with vitamin D deficiency as well as differentiating between intoxication and other hypercalcemic disorders.

Method

- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Analytical sensitivity: 4 ng/mL for $25OHD_2$ and $25OHD_3$
- Analytical specificity: no cross-reaction with vitamin D_2 or D_3; $1\alpha,25(OH)_2D_2$; $1\alpha,25(OH)_2D_3$, calcitriol; $25,26(OH)_2D_3$; $1\alpha(OH)D_2$, doxercalciferol; and $1\alpha(OH)D_3$, alfacalcidiol

Specimen Requirements

0.3 mL room temperature serum
0.15 mL minimum

No additive red top preferred
SST red top acceptable
Overnight (8-12 h) fasting is preferred.

VMA (Vanillylmandelic Acid), Urine

39517X
1710X

Clinical Use

- Diagnose catecholamine-producing tumors

Reference Range

24-Hour urine (39517X)

VMA	mg/24-h
Adults	≤6.0
Children	
3-8 y	≤2.3
9-12 y	≤3.4
13-17 y	≤3.9
Creatinine	g/24 -h
Adults	0.63-2.50
Children	
3-8 y	0.11-0.68
9-12 y	0.17-1.41
13-17 y	0.29-1.87

Random urine (1710X)

VMA	mg/g creat
Adults	1.1-4.1
Children	
Birth-6 mo	5.5-26
7-11 mo	6.1-20
1-2 y	2.5-21
3-8 y	1.7-6.5
9-12 y	1.4-5.1
13-17 y	1.5-3.6
Creatinine	mg/dL
0-6 mo	2-32
7-11 mo	2-36
1-2 y	2-128
3-8 y	2-149
9-12 y	2-183
>12 y	
Males	20-370
Females	20-320

Interpretive Information

- Pheochromocytoma
- Neuroblastoma
- Ganglioblastoma
- Stress

Clinical Background

Urinary vanillylmandelic acid (VMA) is the end product of catecholamine metabolism and reflects catecholamine production by chromaffin cells of the adrenal medulla and the sympathetic nervous system.

Pheochromocytomas are rare tumors of the chromaffin cells located in or near the adrenal glands. These tumors are diagnosed on the basis of elevated levels of urinary metanephrines, urinary VMA, and plasma and/or urine catecholamines. Measurement of homovanillic acid (HVA) has little value in identifying patients with pheochromocytoma but differentiates those with neuroblastoma.

Neuroblastomas are malignant tumors that usually occur before 2 years of age; both VMA and HVA levels help in diagnosing these tumors.

Gangliomas are rare, benign, well-differentiated tumors in young adults and are associated with excess production of catecholamines and their metabolites.

Method

- High-performance liquid chromatography (HPLC), electrochemical detection
- Analytical sensitivity: 0.5 mg/L
- Creatinine concentration also reported

Specimen Requirements

10 mL room temperature aliquot of urine; 5 mL minimum

Collect 24-h urine in 25 mL 6N HCl. Record 24-h volume on vial and request form. Adjust pH of random urine to <3.0 using 6N HCl.

It is preferable for the patient to be off medications for 3 days prior to collection; however, common antihypertensives cause minimal or no interference. Patients should avoid alcohol, coffee, tea, tobacco, nicotine patch, bananas, citrus fruit, and strenuous exercise prior to collection

Test Application

and Interpretation

Test Application and Interpretation

PREFACE

Advances in our understanding of endocrine physiology and pathophysiology and the availability of highly sensitive and specific hormone assays permit increasingly accurate endocrine diagnosis and differential diagnosis. As a result, dynamic tests measuring endocrine gland reserve or autonomy are less often necessary; however, they are still useful in evaluation of hormonal systems that change rapidly, such as growth hormone. Stimulation and suppression tests are still applied to the assessment of selected patients with pituitary and adrenal disorders.

In this test manual, we have attempted to summarize the state of the art with regard to endocrine assay applications, dynamic tests, and their interpretation circa 2007. We hope that this guide provides helpful information for physicians entrusted with the care of patients with known and suspected endocrine disorders.

Test Application and Interpretation

DISORDERS OF ADRENAL FUNCTION

Background Physiology

The hypothalamic-pituitary-adrenal axis (Figure 1) functions as a "closed loop" control system. Adrenocorticotropic hormone (ACTH) stimulates adrenal cortisol production, which, by negative feedback at the hypothalamic and pituitary levels, inhibits corticotropin releasing hormone (CRH) and ACTH release, respectively. CRH is a 41-amino acid, hypothalamic, neurosecretory hormone that stimulates ACTH secretion. Arginine vasopressin (AVP), usually known for its antidiuretic properties, potentiates CRH stimulation of ACTH. ACTH is secreted in a pulsatile and diurnal pattern. The lowest concentrations of both ACTH and cortisol are seen at 6 to 11 PM and the highest concentrations at 6 to 10 AM. The striking diurnal variation in cortisol/ACTH secretion is also associated with a diurnal variation in sensitivity to ACTH suppression by exogenous glucocorticoids, such as dexamethasone. The most sensitive time for suppression is 10 to 11 PM, when as little as 0.125 to 0.25 mg of dexamethasone will suppress the next morning's ACTH surge and increased cortisol production. Figure 2 shows the mean concentrations of cortisol plotted against time of day.

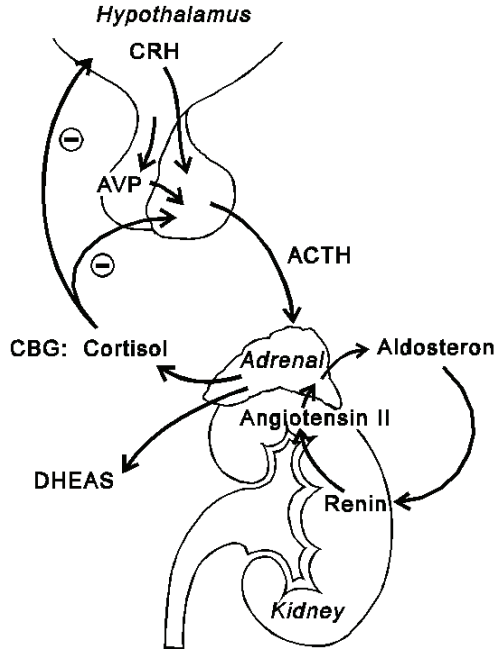

Figure 1. Features of the hypothalamic-pituitary-adrenal axis. CRH, corticotropin releasing hormone; AVP, arginine vasopressin; CBG, cortisol-binding globulin; DHEA-S, dehydroandrostenedione sulfate, an adrenal androgen precursor.

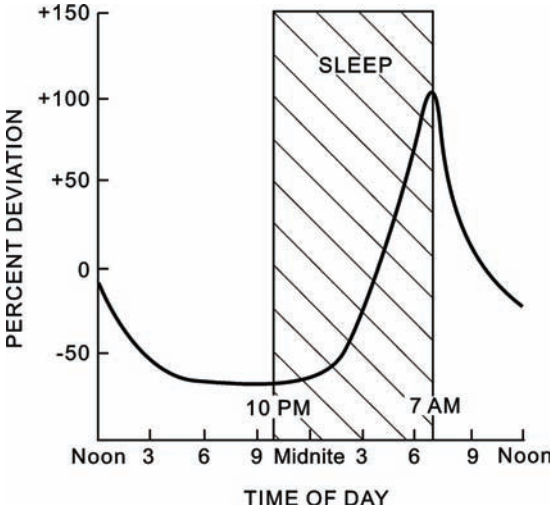

Figure 2. Temporal pattern of plasma cortisol concentrations in normal subjects with regular sleep hours.

The differential diagnosis of adrenal gland dysfunction has been greatly facilitated by availability of sensitive and specific assays for ACTH and adrenal hormones, their precursors, and metabolites. These methods now often allow accurate diagnosis based on single blood samples. Table 1 summarizes the application of commonly available tests for patient diagnosis.

In some instances, diagnosis requires further assessment of pituitary-adrenal reserve or adrenal steroid biosynthetic pathway integrity. In such patients, stimulation of the adrenal glands with ACTH, or suppression of the pituitary-adrenal axis with dexamethasone, provides important supplementary information. Pituitary reserve can be tested with CRH or metyrapone (see section on Dynamic Tests). It is important to recognize that most steroid hormone immunoassays are subject to cross-reaction with closely related steroids. Thus, assays that do not employ extraction or purification of the sample prior to immunoassay may be misleading in diagnosing disorders of enzyme function, such as congenital adrenal hyperplasia.

Steroid hormones and their urinary metabolites can be definitively characterized by gas chromatography/mass spectrometry (GC/MS). Steroid excretion patterns assessed in this way can provide discriminating diagnostic information as a supplement to serum assays. Liquid chromatography, tandem mass spectrometry (LC/MS/MS) technology now is available for highly sensitive and specific measurement of steroid hormones and their metabolites in serum or urine. Quest Diagnostics Nichols Institute has moved from the extraction, chromatography, and radioimmunoassay technology to LC/MS/MS as the next generation platform for steroid hormone assay. There is a high correlation between steroid hormone concentrations measured by extraction, chromatography, and immunoassay and by LC/MS/MS.

Test Application and Interpretation

Table 1. Hormone Changes in Adrenocortical Disorders

Disorder	ACTH	Direct Renin	Cortisol	Aldosterone	Discriminating Precursor
Adrenal insufficiency	↑	↑	N or ↓	N or ↓	NA
Cushing's syndrome*					
Adrenal	↓	↓	↑ †	N or ↓	NA
Pituitary	N or ↑	↓	↑	N or ↓	NA
Ectopic ACTH	↑	↓	↑	↓	NA
ACTH deficiency	N or ↓	N or ↑	↓	N	NA
Glucocorticoid resistance	↑	↓	↑	↓	↑ mineralocorticoids
Congenital adrenal hyperplasia	N or ↑	↑ or ↓	N or ↓	N or ↓	↑ discriminating precursor/product ratio
Primary hyperaldosteronism	N	↓	N	↑	↑ aldosterone to 18-OH corticosterone ratio
Dexamethasone suppressible hyperaldosteronism	N	↓	N	↑	↑ 18-OH cortisol
Apparent mineralocorticoid excess	↓	↓	N	↓	↓ Cortisone to cortisol ratio
Hypoaldosteronism	N	↑	N	↓	↑ 18-OH corticosterone to aldosterone ratio
Pseudohypoaldosteronism	N	↑	N	↑	Non-responsive to aldosterone
Hyporeninemic hypoaldosteronism	N	N or ↓	N	↓	↑ prorenin

N, normal; NA, not applicable.
*24-hour urine cortisol is increased.
†ACTH and cortisol are secreted with striking diurnal changes. Thus, a "normal" 8:00 AM cortisol/ACTH that does not change diurnally can cause Cushing's syndrome.

Abbreviations

ACTH	Adrenocorticotropic hormone
Aldo	Aldosterone
3β-HSD	3β-Hydroxysteroid dehydrogenase
CAH	Congenital adrenal hyperplasia
Compound S	11-Deoxycortisol
CRH	Corticotropin releasing hormone
DHEA	Dehydroepiandrosterone
L-Dopa	Levodopa
17-KGS	17-Ketogenic steroids
17-OHCS	17-Hydroxycorticosteroids
P-450$_{scc}$ (CYP11A1)	Adrenal side-chain cleavage enzyme
P-450$_{c21}$ (CYP21A2)	Adrenal 21-hydroxylase
P-450$_{c17}$ (CYP17)	Adrenal and gonadal 17-hydroxylase
P-450$_{c11}$ (CYP11B1)	Adrenal 11β-hydroxylase
P-450$_{c11}$ase (CYP11B2)	Adrenal aldosterone synthetase

Test Application and Interpretation

Adrenal Insufficiency

Adrenal insufficiency has 2 major causes: ACTH deficiency (secondary adrenal insufficiency) and decreased responsiveness to ACTH (primary adrenal insufficiency). These causes can be further classified as shown in Table 2. With severe primary or secondary adrenal insufficiency, the serum cortisol concentration is very low. However, with partial adrenal insufficiency, basal serum cortisol levels are normal or low, and diurnal rhythmicity may overlap the normal range. ACTH levels, however, are increased. ACTH deficiency is associated with low levels of both cortisol and ACTH. In some instances, particularly in patients with mild deficiency, ACTH stimulation testing may be useful. For primary or secondary disease, the standard 30- or 60-minute ACTH stimulation test is useful. Normal rapid ACTH stimulation test results for infants and children are shown in Tables 3 and 4; test results for adults are shown in Figures 3 and 4 and Table 5. For secondary disease (ACTH deficiency), the CRH test, metyrapone test, or prolonged ACTH test may be helpful in selected patients (see "Dynamic Test Application and Interpretation" section).

Adrenal Biosynthetic Enzyme Deficiencies

Classical Congenital Adrenal Hyperplasia (CAH)

Classical CAH is uniquely manifest in infancy by inefficiency of cortisol synthesis; such inefficiency is caused by genetic mutations that result in deficient activity of 1 of the adrenal biosynthetic enzymes (Figure 1). The most common deficiency is P-450$_{c21}$ (CYP21A2) activity, accounting for 95% of CAH patients. P-450$_{c11}$ (CYP11B1) deficiency accounts for another 4%; 3β-hydroxysteroid dehydrogenase (3β-HSD) and P-450$_{scc}$ (CYP11A1) deficiencies constitute the remainder of cases in infancy. CAH is virilizing in all of these disorders, except P-450$_{scc}$ deficiency, because of the increased production of DHEA or androstenedione induced by ACTH-mediated adrenal hyperactivity in the presence of blocked cortisol synthesis (Figure 5). Newborn screening programs now commonly screen for CYP21A2 deficiency by measuring 17-hydroxyprogesterone levels in filter paper blood samples.

Table 2. Differential Diagnosis of Adrenal Insufficiency

Pituitary dependent	Primary adrenal insufficiency
CRH dependent	Congenital adrenal hyperplasia
Hypothalamic tumors	Congenital adrenal hypoplasia
Idiopathic, acquired	Congenital ACTH unresponsiveness
Congenital	Autoimmune disease
ACTH deficiency, congenital	Infectious diseases
Hypopituitarism	Tuberculosis
Congenital causes	Fungi
Neoplastic	Cytomegalovirus
Craniopharyngioma	Hemorrhage
Infection	Metastatic tumors
Sarcoidosis	Sarcoidosis
Head trauma	Amyloidosis
	Adrenoleukodystrophy

Table 3. Serum Adrenal Steroid Concentrations in Infants. Observed Range Before (B), After (A), and Response (△, range) to Rapid ACTH Test*

Steroid		Premature†		Term
		26-28 wk	34-36 wk	1-6 mo
Pregnenolone	B	260-2100	203-1024	10-150
	A	962-3179	637-1888	110-359
	△	70-2673	162-1685	20-282
Progesterone	B	18-640	–	5-53
	A	52-1348	–	74-200
	△	29-796	–	35-165
17-OH pregnenolone	B	375-3559	559-2906	52-828
	A	2331-11440	831-9760	633-3286
	△	1219-9799	346-8911	229-3104
17-OH progesterone	B	124-841	186-472	13-173
	A	285-1310	334-1725	85-250
	△	50-596	18-1253	52-193
DHEA	B	236-3640	223-3640	26-505
	A	1320-8952	727-7821	67-1453
	△	408-8610	32-7219	28-1343
Androstenedione	B	92-892	90-837	6-78
	A	145-1248	183-1367	21-114
	△	40-718	13-1084	9-76
11-Deoxycortisol	B	110-1376	70-455	10-200
	A	206-2504	81-645	101-392
	△	15-1128	40-190	5-366
Cortisol	B	1-11	3-34	3-22
	A	6-52	16-76	27-50
	△	4-41	6-44	19-41
Deoxycorticosterone	B	20-105	28-78	7-48
	A	44-320	28-95	40-158
	△	17-215	1-67	13-144
Corticosterone	B	235-1108	201-5030	78-2500
	A	1667-8251	2240-11900	2225-4974
	△	1338-8016	2039-10141	1149-4789
18-OH corticosterone	B	10-670	38-779	5-300
	A	35-1500	152-2183	130-465
	△	16-830	114-2183	21-394
Aldosterone	B	5-635	12-736	2-71
	A	13-1046	42-1365	5-166
	△	8-517	28-629	3-123

*Results in ng/dL except cortisol (μg/dL); data from extraction, chromatography, RIA (Ref. 23).
†Samples obtained on postnatal days 2-4.

Table 4. Observed Ranges for Serum Adrenal Steroids in Infants and Children. Values Before (B), After (A), and Response (△) to Rapid ACTH Test

Steroid		<1 y	1-5 y	6-12 y	Pubertal Male	Pubertal Female
Pregnenolone	B	10-137	10-48	15-45	15-84	24-50
	A	49-359	34-135	38-104	33-218	37-149
	△	19-282	4-114	16-73	6-193	9-101
Progesterone	B	5-80	8-64	5-93	6-1286	17-145
	A	74-200	51-233	38-204	32-1069	35-223
	△	35-192	19-192	22-170	0-104	0-192
17-OH pregnenolone	B	13-788	9-98	10-177	19-346	50-516
	A	373-3125	43-702	67-624	84-817	239-1525
	△	200-3000	15-680	60-500	65-750	108-1280
17-OH progesterone	B	11-173	4-114	7-69	12-190	18-220
	A	85-466	50-350	75-218	69-313	80-422
	△	50-275	30-300	50-250	7-281	9-287
DHEA	B	26-500	9-42	11-153	25-400	69-686
	A	18-1100	21-98	34-322	62-509	95-1557
	△	5-600	5-70	20-220	22-386	26-1233
Androstenedione	B	6-78	5-51	7-68	17-151	43-221
	A	21-139	12-68	12-98	2-215	58-319
	△	10-75	5-60	5-60	8-121	9-118
11-Deoxycortisol	B	10-200	7-210	14-136	11-151	15-130
	A	80-390	98-360	95-322	87-283	78-250
	△	5-350	50-280	30-180	35-241	34-233
Cortisol	B	3-23	5-25	5-23	4-15	4-16
	A	32-60	22-40	17-40	15-45	16-35
	△	17-40	5-25	5-20	5-32	7-26
Deoxycorticosterone	B	7-57	4-49	5-34	2-12	4-30
	A	20-157	26-143	19-138	13-63	12-74
	△	26-110	23-135	16-130	10-53	7-43
Corticosterone	B	78-1750	120-2030	155-1365	111-598	115-1219
	A	2225-6505	2150-7540	1775-7500	1723-5100	1472-5060
	△	1140-5120	960-7300	1490-7300	1380-4700	1003-4740
18-OH corticosterone	B	5-310	7-155	10-74	11-82	5-73
	A	67-470	49-370	79-360	69-322	73-1472
	△	22-395	33-333	69-310	58-254	22-1467
Aldosterone	B	2-130	2-37	3-21	2-32	1-14
	A	5-167	13-85	14-50	10-34	10-33
	△	4-122	7-54	4-40	0-22	7-25

Results in ng/dL except cortisol (μg/dL); ACTH 1-24 (250 μg) given as intravenous bolus; age groups ranged from 15-22 children (M and F) except pubertal (n=43, 23M, 20F, Tanner II-V); data from extraction, chromatography, RIA (Ref. 21, 22).

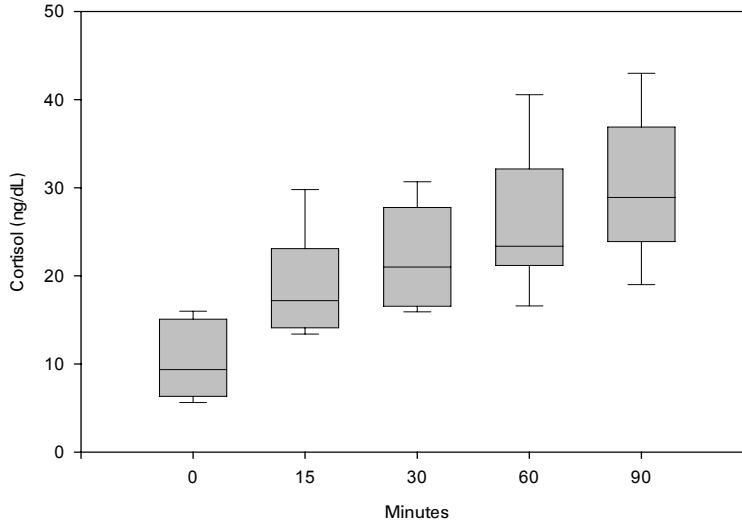

Figure 3. Rapid ACTH stimulation results in normal adults (glucocorticoid pathway); steroid analyses by extraction, chromatography, and radioimmunoassay.

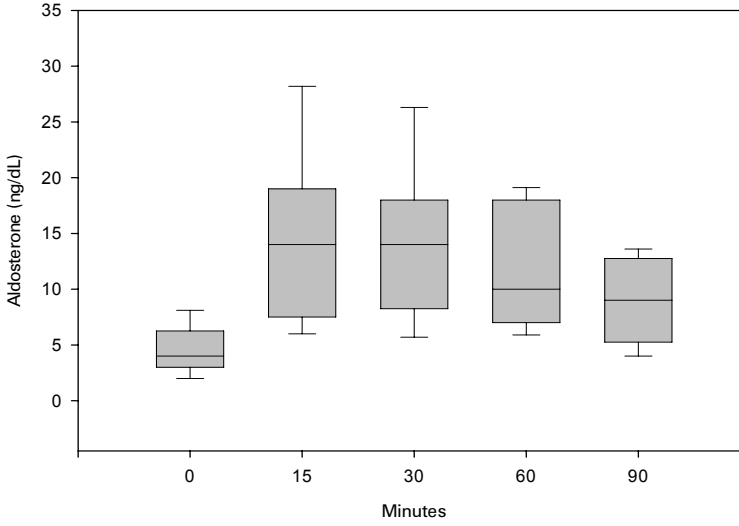

Figure 4. Rapid ACTH stimulation results in normal adults (mineralocorticoid pathway); steroid analyses by extraction, chromatography, and radioimmunoassay.

Table 5. Rapid ACTH Stimulation Results in Normal Adults

Steroid	Range of Blood Levels in ng/dL (Cortisol, µg/dL)				
	0	15 min	30 min	60 min	90 min
Glucocorticoid pathway					
17-Hydroxypregnenolone	29-189	267-856	305-847	293-913	252-846
17-Hydroxyprogesterone	27-122	63-183	74-211	72-187	58-180
Dehydroepiandrosterone	230-955	472-1681	545-1785	545-1846	545-1855
Androstenedione	56-134	69-206	88-271	72-288	98-292
11-Deoxycortisol	21-133	82-232	82-292	82-261	82-246
Cortisol (µg/dL)	5.8-19	13-30	14-36	14-41	18-43
Mineralocorticoid pathway					
Progesterone	5-33	–	11-44	21-44	21-69
Deoxycorticosterone	3-10	–	11-35	14-33	13-30
18-Hydroxycorticosterone	11-46	43-131	45-151	54-161	61-233
Aldosterone	2-9	6-33	5-27	5-20	4-15

Steroid analysis by extraction, chromatography, and radioimmunoassay. Data from Quest Diagnostics Nichols Institute Clinical Correlations Department; from healthy young adults (10 females and 10 males).

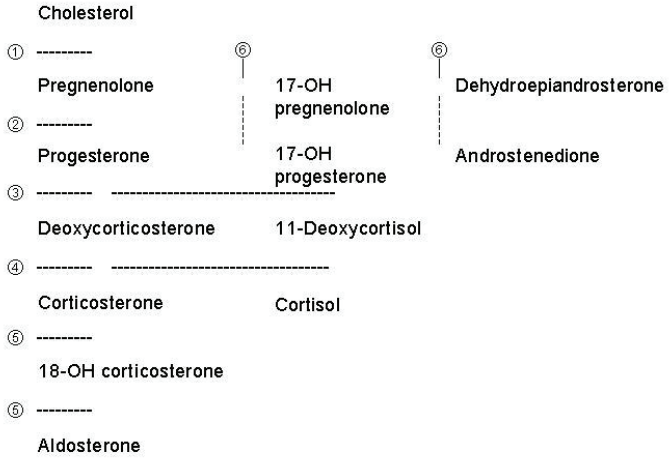

Figure 5. Adrenal gland biosynthetic pathways. The enzymes involved are 1) P-450$_{scc}$ (CYP11A1), 2) 3β-HSD, 3) P-450$_{c21}$ (CYP21A2), 4) P-450$_{c11}$ (CYP11B1), 5) P-450$_{c11}$ase (CYP11B2), and 6) P-450$_{c17}$ (CYP17).

Test Application and Interpretation

The differential diagnosis of congenital virilizing adrenal hyperplasia in infancy includes P-450$_{c21}$, P-450$_{c11}$, and the rare 3β-HSD deficiencies. Diagnosis of these disorders in infancy, and especially in premature infants, is complicated by the normal, transient deficiency of 3β-HSD activity in the fetal adrenal cortex (which resolves during the early weeks and months of life) and by the apparent deficiency of P-450$_{c11}$ activity in premature infants <30 weeks of gestational age. Blockage of the adrenal biosynthetic pathway is manifest by increased enzyme precursor levels or an increased steroid precursor to product ratio imposed by the deficient enzyme activity. Normal baseline and ACTH-stimulated precursor/product ratios for childhood and adolescent ages are shown in Tables 6-8. Tables

Table 6. Normal Ranges in Children for Adrenal Enzyme Precursor/Product Ratios. Values Before (B) and After (A) Rapid ACTH Test*

Precursor / Product Ratios	6 mo - 1 y		1-5 y		6-12 y		Pubertal	
	B	A	B	A	B	A	B	A
3β-HSD								
17-OH pregnenolone / 17-OH progesterone	2-22	2-20	0.3-3.0	0.5-3.3	0.5-6.0	0.3-5.3	0.4-3.4	0.5-6.3
DHEA / Androstenedione	0.8-6.5	1.5-13	0.6-9.0	0.7-7.5	2.0-4.5	1.1-5.8	1.5-4.0	1.8-4.9
P450$_{c17}$								
Pregnenolone / 17-OH pregnenolone	0.1-2.9	0.1-0.5	0.3-3.6	0.2-1.5	0.2-2.8	0.2-0.9	0.1-1.7	0.1-1.0
Progesterone / 17-OH progesterone	0.2-5.2	0.4-1.1	0.2-3.5	0.5-1.5	0.2-2.6	0.2-0.9	0.2-2.2	0.2-1.5
P450$_{c21}$								
Progesterone / Deoxycorticosterone	0.3-7.0	0.9-4.0	0.5-10	0.6-6.0	0.9-8.4	0.9-3.7	1.3-14	1.2-6.6
17-OH progesterone / 11-Deoxycortisol	0.4-3.1	0.5-2.0	0.3-2.1	0.5-1.6	0.2-2.1	0.5-1.6	0.2-3.7	0.4-2.7
P450$_{c11}$								
11-Deoxycortisol / Cortisol	0.8-10	2.4-10	1.0-6.8	3.8-11	1.2-9.0	2.8-9.0	1.8-12	3.4-11
P450$_{c11}$ase								
18-OH corticosterone / Aldosterone	1.3-5.0	2-13	1.2-6.0	1.9-15	2.6-7.1	5-12	2.0-5.7	3.4-13

*Values in $\frac{\text{ng/dL}}{\text{ng/dL}}$ except $\frac{\text{11-deoxycortisol}}{\text{cortisol}} = \frac{\text{ng/dL}}{\mu\text{g/dL}}$.

Measurements by extraction, chromatography, and radioimmunoassay; data from references 21, 22, and Quest Diagnostics Nichols Institute Clinical Correlations.

Table 7. Normal Ranges in Infants for Adrenal Enzyme Precursor/Product Ratios. Values Before (B) and After (A) Rapid ACTH Test*

Precursor / Product Ratios	Premature 26-28 wk		Premature 34-36 wk		Term Infants 1-6 mo	
	B	A	B	A	B	A
3β-HSD						
17-OH pregnenolone / 17-OH progesterone	1.1-5.2	3.6-11	1.8-6.5	3.6-12	2-22	3-20
DHEA / Androstenedione	1.0-8.4	3.4-15	1.6-5.0	2.2-7.8	2.2-6.5	2.8-13
$P450_{c17}$						
Pregnenolone / 17-OH pregnenolone	0.3-0.7	0.3-5.0	0.2-0.7	0.2-1.3	0.1-0.7	0.03-0.3
Progesterone / 17-OH progesterone	0.2-1.8	0.1-1.5	–	–	0.2-2.1	0.4-1.1
$P450_{c21}$						
Progesterone / Deoxycorticosterone	1.6-9.4	1.1-9.8	–	–	0.3-7.0	0.9-4.0
17-OH progesterone / 11-Deoxycortisol	0.4-2.4	0.3-2.1	0.9-4.8	0.8-4.2	0.4-3.1	0.5-2.0
$P450_{c11}$						
11-Deoxycortisol / Cortisol	25-300	10-189	3-115	3-26	0.8-10	2.4-10
$P450_{c11}$ase						
18-OH corticosterone / Aldosterone	1.0-4.5	0.8-2.6	1.1-10	1.2-11	1.3-5.0	2-13

*Values in ng/dL / ng/dL except 11-deoxycortisol / cortisol = ng/dL / µg/dL.
Premature data derived during first week; measurements by extraction, chromatography, and radioimmunoassay; data from references 23, 24.

9 and 10 summarize neonatal urine GC/MS steroid reference ranges. Figures 6-9 illustrate the urine GC/MS steroid precursor-product ratios in normal vs CAH infants with proven 21-hydroxylase or 3β-HSD deficiency. Diagnostic ratios for infants with 21-hydroxylase, 17-hydroxylase, 11β-hydroxylase, and 3β–HSD deficiencies are listed in the "Alphabetical Test Section" (CAH Profile, Neonatal Random Urine). As in childhood and adolescence, normal ratios in adults are

Table 8. Adrenal Enzyme Precursor/Product Ratios in Infants and Children with Documented Deficiencies of Adrenal P-450 Enzymes

Precursor / Product Ratios	P-450$_{c21}$ Type I	P-450$_{c21}$ Type II	P-450$_{c11}$	P-450$_{c17}$	P-450$_{c11}$ase
3β-HSD					
17-OH pregnenolone	0.2	0.2	3	2	*
17-OH progesterone	*	(0.3)	(2)	(1)	*
DHEA	0.7	3	3	2	*
Androstenedione	*	(5)	(0.6)	(7)	*
P450$_{c17}$					
Pregnenolone	0.2	0.7	0.4	15	*
17-OH pregnenolone	*	(0.2)	(0.4)	(35)	*
Progesterone	0.1	0.1	0.8	22	*
17-OH progesterone	*	(0.1)	(0.6)	(16)	*
P450$_{c21}$					
Progesterone	**200**	4	1.2	2	*
Deoxycorticosterone	*	(10)	(0.6)	(2)	*
17-OH progesterone	**250**	**20**	0.1	0.5	4
11-Deoxycortisol	*	(60)	(0.1)	(0.7)	(1.5)
P450$_{c11}$					
11-Deoxycortisol	20	4	**850**	28	7
Cortisol	*	(4)	(350)	(17)	(10)
P450$_{c11}$ase					
18-OH corticosterone	1.0	6	2	**110**	**150**
Aldosterone		(8)	(4)	(120)	(180)

Values in $\frac{\text{ng/dL}}{\text{ng/dL}}$ except $\frac{\text{11-deoxycortisol}}{\text{cortisol}} = \frac{\text{ng/dL}}{\mu\text{g/dL}}$.
Measurements derived from extraction, chromatography, and radioimmunoassay. Ratio values in parentheses are post ACTH stimulation. Ratios were calculated from data published in reference 24, with permission. Bold print indicates discriminating ratios.
*No results available.

similar before and after ACTH and do not exceed 10 for the 3β-HSD, P-450$_{c17}$, and P-450$_{c21}$ enzymes. Normal adult precursor/product ratios for P-450$_{c11}$ and P-450$_{c11}$ase do not exceed 15. Table 8 summarizes the adrenal enzyme precursor/product ratios in infants and children with documented deficiencies of adrenal P-450 enzymes.

Table 9. Pediatric Urine Steroid Reference Ranges (µg/g creat, GC/MS Analysis)

Steroid	LOQ	1 Day Old	2-4 Days Old	>16 Days Old
17α–Hydroxy-pregnanolone	10	30-420	15-200	40-90
15β,17α-Dihydroxy-pregnanolone	20	85-490	25-270	45-80
16α-Hydroxy-DHEA	100	5000-220,000	2000-180,000	200-16,650
Pregnanetriol	2	45-620	20-280	5-70
Tetrahydro-11-deoxycortisol	10	70-670	30-470	15-60
15β,17α-Dihydroxy-pregnenolone	100	4900-43,900	1800-59,000	630-3300
Pregnanetriolone	2	20-280	≤260	≤10
16α-Hydroxy-pregnenolone	50	4400-98,900	3460-150,840	75-10,910
Pregnenetriol	10	≤360	≤390	20-180
Tetrahydrocortisone	100	620-13,160	530-7430	1600-5570
6α-Hydroxytetrahydro-11-deoxycortisol	40	≤120	≤170	<40
Tetrahydro-11-dehydro-corticosterone	150	*	*	320-1490
α-Cortolone	10	25-520	20-380	40-600
β-Cortolone	50	120-1360	150-1175	170-1350
6α-Hydroxytetrahydro-11-dehydrocorticosterone	10	30-540	20-250	10-100

LOQ,, lowest limit of quantitation.
*Prior to 16 days of age, measurement of this steroid is compromised by interference from other fetal steroids.
From Quest Diagnostics Nichols Institute Clinical Correlations Department.

Table 10. Diagnostic Urine Ratios in Normal Neonates and Patients with CAH (GC/MS Analysis)

Steroid Ratio	Age (Days)	Normals n = 59	21-OH n = 32	3β-HSD n = 2[a]	11β-OH n = 2[b]	17-OH n = 1[c]
21-OH Deficiency						
15β-,17α-$(OH)_2$-pregnanolone x 100 / Denominator	1	3.8-17.4	**47.4-400**			
	2-4	1.0-14.4	**116-498**			
	>16	1.0-2.6	**170-1627**	67.1; 150	33.6; 21.4	4.4
17α-OH-pregnanolone x 100 / Denominator	1	2.0-22.0	**29.9-423**			
	2-4	1.0-11.0	**103-974**			
	>16	1.0-4.0	**248-1470**	74.8; 224	36.4; 22.0	8.0
Pregnanetriol x 100 / Denominator	1	2.0-30.0	**36.6-648**			
	2-4	1.0-13.0	**112-286**			
	>16	≤3.0	**76.6-1344**	22.6; 103	16.4; 5.8	4.1
Pregnanetriolone x 100 / Denominator	1	0.7-15.9	**40.6-461**			
	2-4	≤14.5	**45.1-331**			
	>16	≤0.2	**83.3-961**	8.3; 4.6	6.4; 3.4	12.9
17-OH Deficiency						
5α-THA x 100 / Denominator	1	≤8.0	≤55.4			
	2-4	≤11.0	≤7.8			
	>16	≤34.3	≤75.4	ND; 57.0	ND; ND	**50.4**
6α-OH-THA x 100 / Denominator	1	1.0-28.0	ND			
	2-4	1.0-11.0	ND			
	>16	1.0-2.0	ND	ND; ND	NMC; NMC	**29,983**
16α-OH-pregnenolone / 16α-Hydroxy-DHEA	1	0.3-1.3	1.2-4.4			
	2-4	0.1-2.2	1.9-11.4			
	>16	0.2-0.7	1.0-11.7	0.9; 0.4	9.5; 8.1	**156**
11β-OH Deficiency						
THS x 100 / Denominator	1	2.9-25.0	13.6-34.0			
	2-4	1.1-26.0	10.4-49.1			
	>16	0.5-1.4	1.5-136	15.6; 27.0	**366; 97.2**	14.2
6α-OH-THS x 100 / Denominator	1	≤13.0	ND			
	2-4	≤11.0	ND			
	>16	ND	ND	ND; ND	**190,482; 117,723**	ND
***3*β-HSD Deficiency**						
Pregnenetriol x 100 / Denominator	1	≤24.4	≤27.4			
	2-4	≤17.1	≤36.6			
	>16	0.5-2.4	≤287.9	**356; 149**	36.4; 1.2	ND
Pregnenetriol / Pregnanetriolone	1	0.1-10.8	≤0.4			
	2-4	0.2-95.2	≤0.4			
	>16	5.6-28.6	≤1.5	**42.8; 32.5**	2.6; 1.7	ND

Denominator = sum of tetrahydrocortisone, alpha, and beta cortolone; THA, tetrahydro-compound A, tetrahydro-11-dehydrocorticosterone; ND, not detected, numerator level below detectable limit of assay; NMC, not measured due to contamination of numerator component; THS, tetrahydro-substance S, tetrahydro-11-deoxycortisol. From Quest Diagnostics Nichols Institute Clinical Correlations Department (Ref. 18).

[a]One patient; samples collected when 7 and 15 days of age.
[b]Two patients, 14 and 49 days of age.
[c]One 7 day old patient.

Test Application and Interpretation

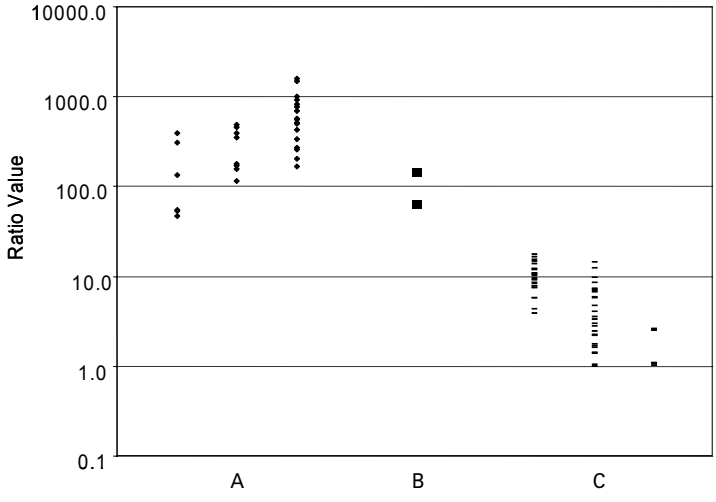

Figure 6. 15β,17α-$(OH)_2$pregnanolone x 100/Denominator.

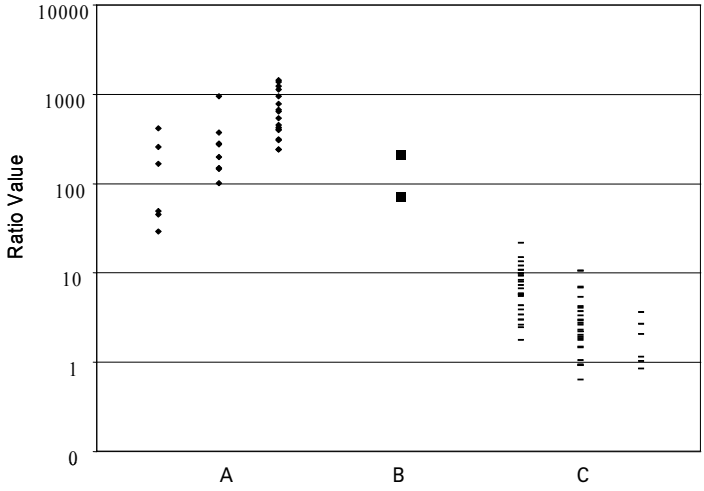

Figure 7. 17α-Hydroxypregnanolone x 100/Denominator.

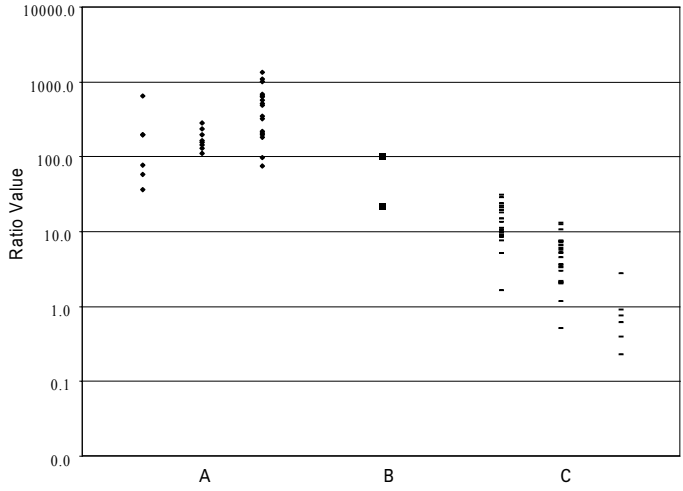

Figure 8. Pregnanetriol x 100/Denominator.

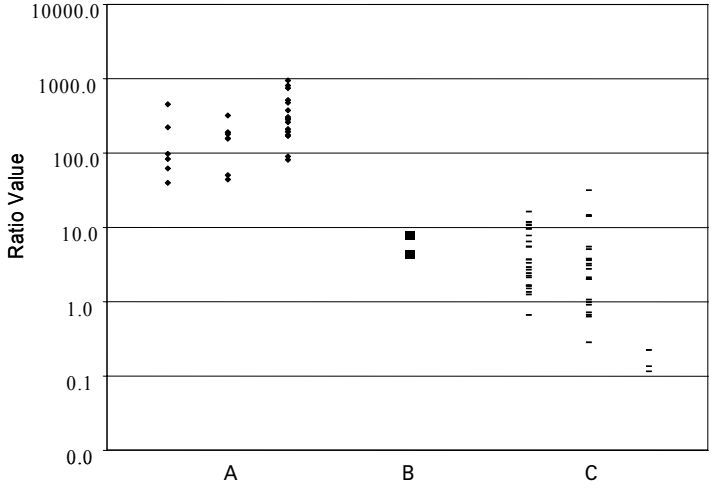

Figure 9. Pregnanetriolone x 100/Denominator.

Figures 6-9. Diagnostic ratios for 21-hydroxylase and 3β-HSD deficiencies using GC/MS analysis. Shown are 4 urinary metabolites relative to the major cortisol metabolites (denominator, sum of tetrahydrocortisone, alpha, and beta cortolone). The patient and normal data points are grouped according to patient/subject age at time of urine collection (day 1, days 2-4, ≥5 days). In patients with 3βHSD deficiency, only the pregnanetriolone ratio is normal. Statistical analysis of data from the 4 ratios (ie, 21-OH patients vs. normal subjects) for the different collection times were P <0.001, <0.001, and 0.001 for 0-1, 2-4, and ≥5d, respectively. **A**, 21-hydroxylase; **B**, 3β-HSD; **C**, normal samples. See Ref. 18.

Test Application and Interpretation

Nonclassical Disease (Partial Deficiency in Steroid Biosynthesis)

Nonclassical, late-onset, partial, or attenuated forms of P-450$_{c21}$, 3β-HSD, and P-450$_{c11}$ adrenal biosynthetic enzyme deficiencies are more common than severe deficiencies producing CAH. The overall prevalence of CAH approximates 1 in 8,000 births but is higher in the Eskimo and Jewish populations. The prevalence of nonclassical P-450$_{c21}$ deficiency varies from 1 in 27 Ashkenazi Jews to 1 in 1,000 in other Caucasians. Nonclassical 3β-HSD and P-450$_{c11}$ deficiencies are much less common, but precise prevalence data are not available. Among Caucasian hyperandrogenic women, nonclassical P-450$_{c21}$ deficiency affects 1% to 2% while nonclassical 3β-HSD deficiency and P-450$_{c11}$ deficiency probably each account for less than 1%. P-450$_{c21}$ gene mutations have been characterized in patients with nonclassical P-450$_{c21}$ enzyme deficiency, but the genetic defects for the 3β-HSD and P-450$_{c11}$ varieties have not been demonstrated. It has been proposed that most hirsute women suspected to have nonclassical 3β-HSD enzyme deficiency have, instead, a nonspecific dysregulation of P-450$_{c17}$ involving the adrenal glands and ovaries.

The common clinical manifestations of nonclassical disease include hirsutism and oligomenorrhea, which result from the excess androgen secretion. Androgenic symptoms usually are mild; clitoromegaly, male habitus, and temporal baldness are infrequent manifestations. Diagnosis is based on elevated precursor and/or precursor-to-product ratios, and detection usually requires ACTH stimulation. Nonclassical disease is confirmed if the discriminating precursor/product ratio is clearly elevated. Stimulated ratios usually exceed 40 in nonclassical P-450$_{c21}$ deficiency patients. In postpubertal females, a morning follicular phase serum 17-hydroxyprogesterone level <200 ng/dL excludes a diagnosis of nonclassical P-450$_{c21}$ enzyme deficiency. ACTH stimulation testing is recommended only when the 8 AM fasting serum 17-hydroxyprogesterone level exceeds this level. Nonclassical P-450$_{c21}$ deficiency is diagnosed by a post-ACTH 17-hydroxyprogesterone level >1,000 ng/dL, a value 4 times the upper normal limit (Figure 3). For the diagnosis of nonclassical 3β-HSD in postpubertal subjects, it is recommended that the post-ACTH 17-hydroxypregnenolone level exceed 1,500 ng/dL, a value 3 SD above the mean of control subjects (Figure 3), and the 17-hydroxypregnenolone/17-hydroxyprogesterone or DHEA/androstenedione ratio should exceed 10, a value exceeding the approximate 3 SD value of control pubertal subjects (Table 6). For the diagnosis of nonclassical P-450$_{c11}$ deficiency in postpubertal patients, the 11-deoxycortisol response to ACTH should exceed 350 ng/dL and the 11-deoxycortisol/cortisol ratio should exceed 15, values approximating 3 SD above the mean for normal subjects (Tables 5 and 6).

Test Application and Interpretation

Cushing's Syndrome

Cushing's syndrome, the clinical manifestations of adrenal cortical hormone excess, can be the result of excessive pituitary or ectopic ACTH production, autonomous adrenal cortical steroid secretion, or exogenous glucocorticoid medication (Table 11). Endogenous Cushing's syndrome is associated with increased 24-hour excretion of urinary cortisol, while administration of synthetic glucocorticoids (eg, prednisone, dexamethasone) is typically accompanied by decreased cortisol excretion, since the adrenal axis is suppressed (Table 12).

If endogenous cortisol hypersecretion is suspected, the simplest approach to diagnosis is collection of a 24-hour urine sample for measurement of free cortisol (with creatinine to assess the completeness of collection). If excessive urine free cortisol is confirmed on two 24-hour urine collections, Cushing's syndrome is usually present, particularly if the value is >200 µg/24-h. Depression, alcohol abuse, and other stresses can also produce lesser degrees of excessive urine free cortisol excretion (pseudo-Cushing's). Alternative approaches to confirm hypercortisolism are to 1) determine the suppressibility of serum cortisol using the overnight dexamethasone suppression test (see "Dynamic Test Application and Interpretation" section), or 2) measure the serum or salivary cortisol level at 11 PM to detect a disrupted circadian rhythm.

Once endogenous hypercortisolism is established, differentiating the cause as pituitary tumor, ectopic neoplasm, or adrenal tumor can be accomplished by plasma ACTH measurement, dynamic testing, and imaging procedures (Table 11). In patients with ACTH-secreting pituitary adenomas (Cushing's disease), plasma ACTH is typically 30 to 150 pg/mL. An exception is Nelson syndrome, in which previous adrenalectomy is associated with increased production of ACTH and growth of a pituitary corticotropinoma. The ectopic ACTH syndrome is often associated with very high plasma ACTH levels, although these may overlap the range of ACTH-secreting pituitary adenomas; ectopic ACTH production is also characterized by secretion of "big" molecular species of ACTH and, rarely, by co-secretion of corticotropin releasing hormone (CRH). Autonomous adrenal cortisol production by a benign or malignant neoplasm is likely if plasma ACTH

Table 11. Differential Diagnosis of Cushing's Syndrome

Pituitary dependent
ACTH adenoma
Adrenotroph hyperplasia
Intermittent Cushing's
Adrenal disease
Adenoma
Carcinoma
Macronodular disease
Micronodular dysplasia
Other (McCune Albright syndrome, Carney syndrome)
Ectopic (cancer-associated) Cushing's
Ectopic ACTH
Ectopic CRH
Pseudo-Cushing's (alcohol-related)
Exogenous glucocorticoid administration (eg, prednisone)

Table 12. Hormone Changes in Cushing's Syndrome

Cause	Plasma ACTH	Plasma Cortisol (PM)	High Dose or Overnight Dexamethasone Suppression
Pituitary-dependent	N or slightly ↑	↑	Yes
Adrenal disease	↓ or undetectable	↑	No
Ectopic Cushing's*	↑↑↑	↑↑	Usually no
Pseudo-Cushing's	N or slightly ↑	N or ↑	Usually yes

*Plasma CRH measurements may also be indicated. ACTH levels in some patients overlap those of pituitary dependent disease.

levels are low or undetectable, although mishandling of plasma samples may lead to misleadingly low ACTH concentrations due to hormone degradation.

Imaging of the pituitary and/or adrenal glands is usually best deferred until a biochemical diagnosis has been established, because false-negative and false-positive findings are common. Only 50% of patients with surgically proven ACTH-secreting pituitary adenomas have a lesion that is visualized by MRI. Conversely, up to 20% of normal individuals may have radiological findings consistent with a pituitary adenoma, which may be an entirely incidental lesion. Similarly, as many as 3% of normal individuals may have adrenal cortical masses seen on CT or MR imaging. The most definitive contemporary test to distinguish pituitary from ectopic ACTH hypersecretion, and to localize pituitary tumors, is sampling of the pituitary venous effluent by bilateral inferior petrosal sinus (IPS) catheterization. Measurement of ACTH in simultaneously obtained IPS and peripheral venous samples can identify the true source of endogenous ACTH production, particularly when additional samples are obtained after CRH stimulation (see "Dynamic Test Application and Interpretation" section).

Renin-Angiotensin — Aldosterone System

Background Physiology

The renin-angiotensin aldosterone system (RAAS) modulates, in association with vasopressin, body salt and water metabolism (Figure 1). Renal sodium excretion is regulated by the RAAS to maintain sodium homeostasis relative to salt intake. Renal renin secretion is stimulated by decreased blood volume and decreased renal perfusion pressure. Renin, in turn, stimulates cleavage of circulating angiotensinogen substrate to produce angiotensin I, an inactive decapeptide. Angiotensin converting enzyme (ACE) converts angiotensin I to angiotensin II, an octapeptide, and angiotensin III, a heptapeptide. Both angiotensin II and III stimulate aldosterone secretion via the type I angiotensin receptor. Angiotensin II also acts as a vasoconstrictor, and the net effect of the increase in renin and angiotensin II levels is expansion of extracellular and blood volume and increased blood pressure. Increased blood volume inhibits renin, reduces angiotension II and III levels, and decreases aldosterone secretion, resulting in increased sodium excretion.

Test Application and Interpretation

Vasopressin (antidiuretic hormone or ADH) modulates plasma osmolality, within narrow limits, by regulating renal free water excretion (urine osmolality). Increased serum sodium concentration (increased osmolality) stimulates pituitary ADH secretion which increases renal tubular water reabsorption to expand volume and reduce extracellular sodium concentration. Decreased sodium concentration inhibits ADH secretion decreasing urine osmolality, increasing free water excretion, and increasing serum sodium concentration.

Thus, the kidney plays an essential role in sodium and water balance and blood pressure. Several genes are involved in renal tubular sodium reabsorption from the glomerular filtrate, and mutations in these genes have been associated with the rare Mendelian disorders of body sodium homeostasis (Ref. 8-9). These disorders and the prominent phenotypic manifestations are summarized in Table 13. Although rare, these disorders highlight the important relationship between salt intake, the RAAS, and blood pressure. Attempts to define the more subtle genetic alterations in sodium homeostasis predisposing to hypertension have provided mixed results, but positive correlations of hypertension with expression of the *AGT* gene for angiotensinogen and the *ENaC* gene have been demonstrated in humans and in animal models.

Assessment of Disorders of the RAAS

The key elements of the RAAS (renin and aldosterone) can be measured in blood. The tests are useful in evaluating secondary causes of hypertension, such as renovascular hypertension and primary aldosteronism (Figure 1). In some circumstances, a knowledge of the renin status in essential hypertension can be useful in determining therapy, since patients with high-renin hypertension respond better to renin-lowering agents, while low-renin cases may be better treated with diuretics.

Table 13. Heritable Disorders of Renal Sodium Handling

Disorder	Hypertension	Salt Wasting	Aldo	Renin	K^+	Genes Involved	Inheritance Pattern
Liddle syndrome	Yes	No	↓	↓	↓	*ENaC*	GOF dominant mutations
Gordon syndrome	Yes	No	↓	↓	↑	*WNK*	LOF mutations increase *NCCT* activity
Pseudohypo-aldosteronism	No	Yes	↑	↑	↑	*ENaC*	LOF dominant or recessive mutations
Bartter syndrome	No	Yes	↑	↑	↓	*NKCC2* *ROMK2* *CLCNKB* Barttin	LOF recessive mutations*
Gitelman syndrome	No	Yes	↑	↑	↓	*NCCT*	LOF recessive mutations

Aldo, aldosterone; GOF, gain of function; LOF, loss of function; *ENaC*, epithelial sodium channel; *WNK*, with no lysine potassium kinase; *NKCC2*, sodium-potassium-chloride cotransporter; *ROMK2*, inward rectifier outer medullary potassium channel; *CLCNKB*, basolateral chloride channel; Barttin, cofactor for chloride channel kidney B (*CLC-KB*); *NCCT*, sodium chloride cotransporter.

**ROMK2* or *CLCNKB* LOF mutations lead to decreased *NKCC2* activity.

Test Application and Interpretation

Renin can be measured in 2 types of assays. In the classical method, the level of renin is determined by generating angiotensin I during incubation with angiotensinogen and measuring the angiotensin by RIA. This assay is called plasma renin activity (PRA). The renin direct assay is a newly developed procedure that utilizes an antibody directed to the renin molecule itself. The concentration of renin, rather than renin activity, is measured.

Renin measurements should be made in plasma under controlled or known conditions of dietary sodium intake and body position. A diurnal variation is normally present and samples are typically obtained at 7 to 9 AM. A low-salt diet (<40 mEq/d) and diuretics stimulate renin and aldosterone, while a high-salt diet (>150 mEq/d) suppresses the system. If there is doubt about the general sodium status in the patient, a 24-hour urine measurement of sodium excretion is useful for interpretation. Aldosterone is regulated by the renin-angiotensin system but may be autonomously secreted by an adrenal tumor. In the former situation, renin and aldosterone parallel each other. In the case of primary aldosteronism, renin is suppressed and aldosterone inappropriately increased.

The measurement of renin and aldosterone, in association with serum potassium concentration, provides important differential diagnostic information for the assessment of disorders of the renin-aldosterone axis (Table 14). The effect of mineralocorticoids (ie, increase renal potassium excretion in exchange for reabsorbed sodium) tends to reduce serum potassium levels in hypermineralocorticoid states. Serum potassium concentrations are increased in

Table 14. Assessment of Disorders of the Renin-Aldosterone System

Disorder	Renin	Aldosterone	K^+ (Serum)
Primary aldosteronism	↓	↑	↓
Renin secreting tumor	↑	↑	N or ↓
Pseudohyperaldosteronism (apparent mineralocorticoid excess, AME)	↓	↓	↓
Dexamethasone-suppressible hyperaldosteronism	↓	↑	↓
Hypoaldosteronism (adrenal)	↑	N or ↓	↑
Addison's disease	↑	↓	↑
Hyporeninemic hypoaldosteronism	↓	↓ or N	↑
Mineralocorticoid resistance (pseudohypoaldosteronism)	↑	↑	↑
Bartter syndrome	↑	↑	↓
Renovascular hypertension	N or ↑	N or ↑	N or ↓
Diuretic medication	↑	↑	N or ↓
Congestive heart failure	↑	↑	N or ↓
Liver disease	↑	↑	N or ↓
Nephrosis	↑	↑	N or ↓

states of deficient mineralocorticoid production or action. Patients with secondary hyperaldosteronism caused by congestive heart failure, liver disease, or nephrosis may manifest hypokalemia, but these states are clinically evident. In Bartter syndrome, hyperaldosteronism is caused by juxtaglomerular hyperplasia and is associated with hypokalemic alkalosis in the absence of hypertension or edema.

Primary Aldosteronism

Primary aldosteronism is an uncommon, but usually curable, cause of hypertension. Since aldosterone is the major regulator of potassium balance, hyperaldosteronism is associated with potassium wasting. One marker of the disorder is hypertension with unexplained hypokalemia (and alkalosis). Screening hypertensive patients for hypokalemia should be accomplished prior to the use of antihypertensive therapy, especially diuretics. Hypokalemia per se is normally associated with low aldosterone levels, so the combination of an inappropriate serum aldosterone (>15 ng/dL) and a low renin (<0.2 ng/dL) is diagnostic in the presence of hypokalemia. An elevated (>20) aldosterone/PRA ratio, or an aldosterone/direct renin ratio >5, also is useful in the diagnosis of primary aldosteronism (see Ref. 20). Two maneuvers are sometimes useful to confirm the diagnosis. An infusion of saline or administration of mineralocorticoid (Florinef®, 0.4 mg/d x 2 days) will in each case reduce plasma aldosterone to <5 ng/dL.

Two etiologies are recognized. Conn syndrome is caused by an aldosterone-secreting adrenal adenoma and is characterized by elevated levels of the precursor 18-hydroxycorticosterone with values >50 ng/dL. Less often (20%), the idiopathic form is caused by unexplained bilateral hypersecretion of aldosterone, in which case precursor levels are normal. The diagnosis of suspected primary hyperaldosteronism in patients with hypertension and hypokalemia requires measurements of serum and urine aldosterone and plasma renin (or renin activity) concentrations. 18-Hydroxycorticosterone and 18-hydroxycortisol levels in serum and urine are increased in patients with adrenal adenoma, and levels may be useful to differentiate adenoma from idiopathic hyperplasia. Serum 18-hydroxycortisol levels in >300 ng/dL or 18-hydroxycorticosterone levels >100 ng/dL are suggestive of adrenal adenoma. Diagnosis in some cases may be enhanced by aldosterone suppression testing using saline or fluorohydrocortisone (see "Dynamic Test Application and Interpretation" section). CT or MRI scans identify most adrenal tumors. Rarely, bilateral adrenal vein catheterization is utilized to determine whether the disorder is unilateral or bilateral. Recent reports emphasize the importance of diagnosing primary aldosteronism, since in addition to the hypertension, aldosterone excess leads to a cardiomyopathy and increased incidence of heart failure.

Renovascular Hypertension

Renovascular hypertension is a relatively common cause of primary hypertension and is more common in the elderly (atherosclerosis) and in a cohort of young women (fibronodular). Hypokalemia is observed only in the more severe cases. Suspicion and either a family history (fibronodular) or sudden development of hypertension in an older patient may be the only clues. Peripheral renin is

usually, but not invariably, increased. Aldosterone levels may be either normal or increased. Vascular studies are the key to diagnosis. The best indicator of a positive response to vascular surgery is demonstration of increased renin or renin activity levels (x2) from the ischemic kidney.

Dexamethasone-suppressible Hyperaldosteronism

Dexamethasone-suppressible hyperaldosteronism is a rare form of hypertension characterized by high levels of aldosterone, suppressed renin (or PRA), and a dominant mode of inheritance. Chronic ACTH administration stimulates a continued rise in aldosterone concentration; dexamethasone administration produces a rapid and complete suppression of serum aldosterone. Urinary excretion of 18-hydroxycortisol is increased. The pathogenesis appears to be abnormal regulation of aldosterone secretion by ACTH, perhaps due to intergenic gene promotor sequence recombination such that ACTH simulates transcription of the P-450$_{c11}$ isozyme normally active in the zona glomerulosa.

Pseudohyperaldosteronism

Pseudohyperaldosteronism, a syndrome of apparent mineralocorticoid excess (AME), was first described in children with hypertension and hypokalemia. In adults, the syndrome has been associated with licorice ingestion and Cushing's syndrome. The mechanism has recently been discovered. Adjacent to the mineralocorticoid receptor is the enzyme 11β-hydroxysteroid dehydrogenase. This enzyme normally protects the aldosterone receptor from cortisol, converting cortisol to cortisone. In AME or after heavy licorice ingestion, the enzyme is inactive and cortisol is free to act as a mineralocorticoid. In these patients, both renin and aldosterone are suppressed. Diagnosis can be confirmed by measuring urine cortisol and cortisone metabolites (THF/THE).

Adrenal Diagnosis via Urine GC/MS Metabolite Analysis

Gas chromatography/mass spectrometry (GC/MS) of urinary adrenal steroids offers a useful supplement to serum precursor/product analysis of adrenal steroid enzymatic abnormalities. This approach allows comprehensive precursor/product and pattern analysis of adrenal steroid excretion using small-volume (5-mL) random urine samples. Production data can be estimated from analysis of 24-hour or other timed-interval urine collections. In newborn infants, urine can be collected in a clean diaper and squeezed out into a test tube. Urinary steroids are measured with the methods described by Shackleton or Ulick, et al (Ref. 27, 28). The free and conjugated steroids are first extracted from urine, whereupon the glucuronides and sulfates are cleaved by enzyme hydrolysis. The freed steroids are re-extracted, chemically derivatized to render them heat stable, and separated by fused-silica gas chromatography prior to mass spectrometric quantitation. Table 15 summarizes the measured urinary steroids and their abbreviations. Table 10 summarizes normal and abnormal urine steroid metabolite precursor/product ratios in neonates, children, and adolescents. Normative data for urinary steroid metabolite excretion in adults are shown in Table 16. Table 17 summarizes normal and abnormal steroid precursor/product ratios for the assessment of adrenal biosynthetic enzyme deficiencies in adolescents and adults.

Table 15. Urinary Steroid Analyte Abbreviations

Abbreviations	Trivial Name
AN	Andosterone
ET	Etiocholanolone
DHEA	Dehydroepiandrosterone
5-AD-17β	5-Androstene-3β,17β-diol
11-OXO-ET	11-Oxo-etiocholanolone
17α-HP	17α-Hydroxypregnanolone
11β-OH-AN	11β-Hydroxy-androsterone
11β-OH-ET	11β-Hydroxy-etiocholanolone
16α-OH-DHEA	16α-HydroxyDHEA
PD	Pregnanediol
PT	Pregnanetriol
5-AT	5-Androstene-3β, 16α, 17β-triol
THS	Tetrahydrosubstance S
Estriol	Estriol
PT'ONE	11-Oxo-pregnanetriol
5-PT	5-Pregnene-3β, 17α, 20α-triol
THE	Tetrahydrocortisone
THA	Tetrahydro-11-dehydrocorticosterone
THB	Tetrahydrocorticosterone
5α-THB	5α-Tetrahydrocorticosterone
THF	Tetrahydrocortisol
5α-THF	5α-Tetrahydrocortisol
α-Cortolone	α-Cortolone
THALDO	Tetrahydroaldosterone
β-Cortolone	β-Cortolone
α-Cortol	α-Cortol
Cortisol	Cortisol
11-OXO-AN	11-Oxoandrosterone
18-OXO-THF	18-Oxotetrahydrocortisol
β-Cortol	β-Cortol
Cortisone	Cortisone

The steroids are listed approximately in order of their elution from the gas chromatography column.

Test Application and Interpretation

Table 16. Urinary Steroid Excretion Normal Values (n = 98)

Metabolites	Men µg/24-h Range	Women µg/24-h Range
AN	320-5400	240-2300
ET	430-3300	245-2300
DHEA	21-2710	21-2710
5-AD-17β	17-485	7-175
11-OXO-ET	78-1165	78-1165
17α-HP	59-470	16-460
11β-OH-AN	195-1500	130-900
11β-OH-ET	14-680	14-680
16α-OH-DHEA	81-2600	42-1040
PD	49-385	57-4540
PT	71-1000	47-790
5-AT	95-1320	45-680
THS	1.8-63.5	1.8-63.5
Estriol	0.72-8.17	0.35-16.77
PT'ONE	0.2-26.4	0.9-13.4
5-PT	10-480	47-210
THE	445-5960	330-3430
THA	36-270	36-270
THB	36-245	36-245
5α-THB	37-440	37-440
THF	280-2820	210-1680
5α-THF	100-2660	100-1250
α-Cortolone	47-1400	90-1100
THALDO	6-79	6-79
β-Cortolone	8-560	10-220
α-Cortol	30-490	25-215
Cortisol	7-185	13-115
11-OXO-AN	15-111	8-87
18-OXO-THF	≤42	≤10
β-Cortol	8-340	17-215
Cortisone	19-245	24-160

Data developed by Quest Diagnostics Nichols Institute Clinical Correlations Department using a sample of 48 females and 50 males. All were apparently healthy, non-medicated employees aged 18 to 50 years.

Table 17. Normal Ranges for Urinary Adrenal Enzyme Precursor/Product Ratios in Adolescents and Adults (GC/MS Analysis)

Precursor / Product Ratios	Normal		Affected	
	Male	Female		
3β-HSD				
DHEA / THE + THR + 5α-THF	0.05 0.01-0.27	0.10 0.01-0.60	>5	
5PT / THE + THF + 5α-THF	0.05 0.01-0.1	0.05 0.02-0.08	>5	
AN + ET / THE + THF + 5α-THF	0.66 0.4-1.2	0.60 0.4-1.0	>5	
$P450_{c17}$				
THA + THB + 5α-THB / THE + THF + 5α-THF	0.10 0.06-0.2	0.10 0.05-0.2	>20	
AN + ET / THA + THB + 5α-THB	0.17 0.04-0.30	0.15 0.08-0.25	>20	
$P450_{c21}$			Classic	Late Onset
17HP / THE + THF + 5α-THF	0.04 0.01-0.12	0.03 0.01-0.10	>5	0.17-1.0
PT / THE + THF + 5α-THF	0.10 0.06-0.12	0.10 0.02-0.15	>5	0.25-2.0
P'TONE / THE + THF + 5α-THF	0.003 0.002-0.008	0.004 0.001-0.005	>5	0.08-0.5
$P450_{c11}$				
AN + ET / 11β-OH-AN + 11β-OH-ET	3.0 1.0-4.3	4.1 2.3-4.5	>10	
THS / THE + THF + 5α-THF	0.01 0.006-0.036	0.01 0.005-0.022	>7	
$P450_{c11}$ase				
18-OH-THA / THALDO	3.2 2.6-5.6	2.6 1.7-5.0	>10	
THA + THB + 5α-THB (or 6α-OH-THA) / THE + THF + 5α-THF	0.10 0.06-0.2	0.10 0.05-0.2	>1.0	
THA + THB + 5α-THB (or 6α-OH-THA) / THALDO	20 3.6-22	15 10-22	>50	

From Ref. 8, 29.

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DISORDERS OF ANTERIOR PITUITARY FUNCTION

Background Physiology

The hypothalamic-pituitary complex (Figure 1) is the central control system for most of the important endocrine glands. It functions as a neuroendocrine transducer in order to translate neural system output into endocrine hormone secretion. The hypothalamus is comprised of multiple cell clusters, or nuclei, that regulate synthesis and secretion of a variety of neurotransmitters and hormone releasing factors, including corticotropin releasing hormone (CRH), growth hormone releasing hormone (GHRH), gonadotropin releasing hormone (GNRH), thyrotropin releasing hormone (TRH), dopamine \(DA), and somatostatin (SS), which acts as a release inhibiting factor. The pituitary portal vascular system transports these factors to the anterior pituitary where production/secretion of adrenocorticotropic hormone (ACTH), growth hormone (GH), luteinizing/follicle stimulating hormones (LH/FSH), thyroid stimulating hormone (TSH), and prolactin (PRL) are regulated by the corticotropic, somatotropic, gonadotropic, thyrotropic, and lactotropic cells comprising the anterior pituitary tissue mass. The releasing hormones bind to and activate their respective receptors (CRHR, GHRHR, GNRHR, TRHR, SSR, DAR) in the tropic cells, thereby regulating synthesis and secretion of the anterior pituitary hormones. Arginine vasopressin (AVP) and oxytocin (OT) are produced by the hypothalamic magnocellular neurons whose axons terminate in the posterior pituitary wherein the hormones are stored and secreted. Regulation of PRL secretion is more complex and involves stimulation by TRH, vasoactive

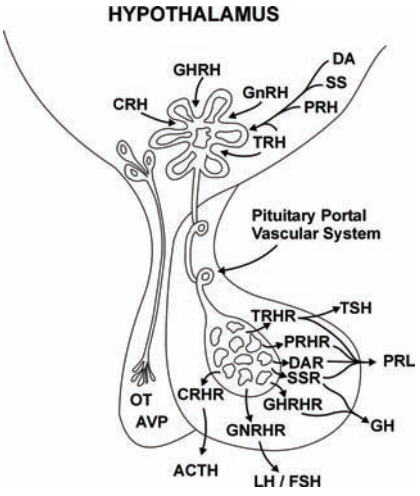

Figure 1. The hypothalamic-pituitary neuroendocrine transducer system functions to translate neuronal output into endocrine hormonal activity. The pituitary portal vascular system transports the releasing hormones dopamine (DA) and somatostatin (SS) to the anterior pituitary where they bind to their cognate receptors to modulate their respective peripheral endocrine organs.

intestinal peptide (VIP), OT (via a short portal system), pituitary adenylate cyclase-activating peptide (PACAP), and galanin and inhibition by DA. GH secretory control also is complex and involves stimulation by GHRH and inhibition by SS. The regulation of these factors within the hypothalamus involves a variety of stimuli (see "Disorders of the Growth Hormone Insulin-like Growth Factor Axis").

A cascade of homeodomain proteins and transcription factors is involved in hypothalamic-pituitary embryogenesis and function (Figure 2). Mutations of the homeobox genes sonic hedgehog (*SHH*), *ZIC1*, and *SIX3* have been described in patients with holoprosencephaly. *HESXI* mutations have been identified in siblings with septo-optic dysplasia in association with midline brain defects and pituitary hypoplasia. *GLI3* mutations have been associated with the Pallister-Hall syndrome involving hypothalamic dysgenesis and hypopituitarism. Transcription factors participating in early pituitary development include the Rathke Pouch gene (*RPX*), *PITX*, and *LHX* genes. Genes participating in later stages of pituitary development, *PROP1* and *PITI*, program development and function of the pituitary cells producing GH, TSH, and PRL.

Hypopituitarism

Hypopituitarism refers to deficiency of 1 or more of the pituitary hormones. ACTH, TSH, gonadotropin, and GH deficiencies are reviewed in separate sections. Multiple pituitary hormone deficiencies are associated with combined manifestations of GH deficiency, hypothyroidism, adrenal insufficiency, and/or hypogonadism. The causes of hypopituitarism are summarized in Table 1. Mutations of the homeobox genes sonic hedgehog (*SHH*), *ZIC1*, and *SIX3* have been identified in patients with holoprosencephaly and *HESX-1* mutations in association with septo-optic dysplasia. *GLI3* defects have been described in the Pallister-Hall syndrome associated with hypothalamic disorganization and hypopituitarism (Figure 2).

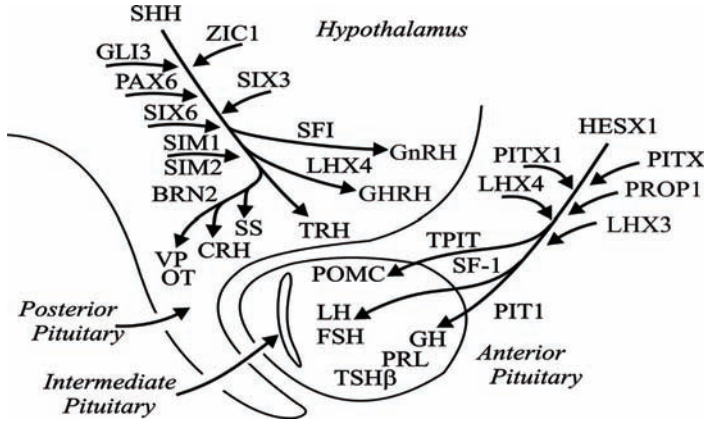

Figure 2. A cartoon displaying the cascades of homeodomain proteins and transcription factors mediating hypothalamic-pituitary embryonic development. See text for details.

Test Application and Interpretation

Mutation of the *TRHR* gene, the *GHRHR* gene, and the *GNRHR* gene has been reported in association with isolated TSH, GH, and gonadotropin hormone deficiencies, respectively (Figure 1). TRH and GHRH deficiencies are usually diagnosed during infancy and childhood because of their profound effect on growth. GNRH deficiency (Kallmann's syndrome) usually is not recognized prior to puberty. This deficiency can be due to mutations in the X-linked *Kal-1* gene or the autosomal Fgf1 receptor gene. Other forms of idiopathic hypogonadotropic hypogonadism are due to mutations in the GP54 receptor gene which may play a role in the timing of puberty. This is discussed in more detail in the gonadal section.

A variety of mutations in pituitary transcription factor genes and pituitary hormone genes have been characterized in hypopituitary patients. The phenotypic features of patients with pituitary transcription factor gene mutations are summarized in Table 2 and are discussed further in related sections.

The pituitary hormones are easily measured in peripheral blood. Hypothalamic hormones are not secreted into peripheral blood, but can be detected in blood where they are derived from ectopic tissue sources. Quantification of selected hypothalamic hormones in peripheral blood can be useful in the diagnosis of patients with excessive pituitary hormone secretion caused by ectopic hypothalamic hormone production from extrapituitary tumors.

Table 1. Causes of Hypopituitarism

Developmental anomalies	Vascular events
Basal encephalocele	Postpartum (Sheehan necrosis)
Holoprosencephaly	Internal carotid aneurysm
Median facial anomalies	Inflammatory diseases
Septo-optic dysplasia	Encephalitis
Pituitary hypoplasia	Meningitis, bacterial
Congenital hormone deficiencies	Granulomatous disease
Hypothalamic	Histiocytosis X
TRH	Autoimmune hypophysitis
GHRH	Destructive lesions
LHRH (Kallmann's syndrome)	Intraventricular hemorrhage
Isolated pituitary hormone deficiencies	Surgery
ACTH	Radiation
TSH	Trauma
GH	Neoplasia
LH, FSH	Hypothalamic tumors
Multiple pituitary hormone deficiencies	Pituitary adenomas
PIT1 mutation	Metastatic carcinoma
PROP1 mutation	Pinealoma
HESX1 mutation	Craniopharyngioma
LHX3 and *LHX4* mutation	

Table 2. Phenotypic Features of Patients with Pituitary Trancription Factor Gene Mutations

	Gene Mutations					
	PROP1	*PIT1*	*LHX3*	*LHX4*	*HESX1*	*TPIT*
GH	D	A	A	D	D	N
Prolactin	D	A	A	V	N	N
TSH	D	D	A	V	V	N
LH/FSH	D	N	A	N	N	N
ACTH	V	N	N	V	N	D
Post pituitary	N	N	N	E	E	N

D, decreased; A, absent; N, normal; V, variable; E, ectopic.
Modified from Parks JS, Brown MR. Ref. 7.

Pituitary Adenomas

General Features

Adenomas of the anterior pituitary gland are relatively common and are variable with regard to their cell of origin and function. Incidental pituitary adenomas have been documented in up to 20% of adult humans at autopsy. Most of these do not produce signs or symptoms. The relative prevalence of the clinically significant pituitary adenomas is summarized in Table 3. The pituitary adenomas are classified by their hormonal secretion(s), and most are characterized by the clinical effects of the excessive hormone production (Table 4). The most common secreting pituitary adenoma is the prolactin-secreting prolactinoma. The least common of the functional varieties are the gonadotropinomas and thyrotropinomas. Most gonadotroph-derived adenomas, also referred to as a chromophobe adenoma, are nonsecreting and present with compressive symptoms associated with multiple hormone deficiencies, including hypogonadism or mild elevations in prolactin. Most of these nonsecreting tumors show immunostaining for 1 or more hormones, including the alpha subunit, LH beta, FSH beta, and, less frequently, TSH beta subunits, and ACTH. Some of these tumors secrete alpha, LH beta, and FSH beta subunits which are detectable in serum and can be used as tumor markers. LH, FSH, and alpha subunit respond paradoxically to TRH stimulation. Thyrotrope secreting tumors lead to hyperthyroidism with an elevated or inappropriately normal TSH. The application of hormone assays to pituitary tumor characterization is summarized in Table 4.

Table 3. Relative Prevalence (%) of Pituitary Adenomas*

	Prevalence (%)
Prolactinomas	29
GH-producing	15
GH + prolactin-producing	8
Corticotroph adenomas	16
Gonadotropinomas	7
Thyrotropinomas	1
Null cell adenomas	27

*From Melmed S, Kleinberg D (Ref 2).

Table 4. Hormone Products Associated with Pituitary Adenomas

Hormone or Precursor	Pro-lactinoma	Acro-megaly	Cushing's Disease	Thyro-tropinoma	Gonado-tropinoma	Non-secretory Adenoma
ACTH			√			
GH		√				
PRL	√					
TSH				√		
LH					√	
FSH					√	
Alpha Subunit				√	√	√
Lipoprotein			√			
Endorphin			√			
Chromogranin	√	√	√	√	√	√
IGF-I		√				
IGFBP-3		√				

Prolactinoma

Serum prolactin levels normally are <15 ng/mL in males and <25 ng/mL in females. Causes of hyperprolactinemia are shown in Table 5.

Table 5. Causes of Hyperprolactinemia

Physiologic
Newborn
Pregnancy
Lactation
Intracranial disease
Hypothalamic disorders
Pituitary stalk disorders
Pituitary adenoma
Idiopathic hyperprolactinemia
Chest wall stimulation
Herpes zoster
Breast infection or tumor
Surgical or accidental traumas
Breast stimulation
Drugs (eg, phenothiazines, metoclopramide)
Miscellaneous
Hypothyroidism
Chronic renal failure
Big/macroprolactinemia

Test Application and Interpretation

A prolactinoma usually is associated with clinical evidence of gonadal dysfunction and/or symptoms related to a pituitary mass effect. Galactorrhea is present in 30% to 80% of women with prolactinomas but is uncommon in men. Basal blood samples are adequate to diagnose hyperprolactinemia. Stimulation testing usually is not required and does not aid in differential diagnosis of prolactinoma. Borderline hyperprolactinemia is best confirmed by measurements in single samples collected on 3 separate days or in 3 separate samples collected at 30-minute intervals via indwelling catheter. Prolactin (PRL) is a stress hormone, and stress should be avoided when sampling blood. Postprandial and post-breast examination sampling should also be avoided. Most prolactinomas are associated with serum PRL levels >100 ng/mL, while levels in drug-induced hyperprolactinemia tend to be lower. Mild elevation of prolactin of <100 ng/mL is also seen in stalk compression due to pituitary or other parasellar masses. The stalk compression relieves the lactotrophs from the normal inhibitory dopaminergic tone. The diagnosis of prolactinoma is confirmed in patients without other obvious causes of hyperprolactinemia by pituitary imaging techniques (Table 5). PRL exists in a variety of forms: the 26 kd monomer, the 40-60 kd dimeric or "big" prolactin, and the >100 kd macroprolactin, which is the monomer complexed with IgG. Macroprolactin is biologically inactive but cross-reacts in several PRL immunoassays. Since the presence of macroprolactin may lead to an overestimation of the biologically active form of PRL, macroprolactin should be specifically measured in patients with apparent hyperprolactinemia that is not consistent with the clinical presentation.

Acromegaly

Acromegaly with or without increased prolactin secretion accounts for some 15% of clinically significant pituitary adenomas. The prevalence ranges from 38 to 69 cases per million population. Clinical features are related to sustained over secretion of GH and IGF-I. The primary pituitary somatotroph adenomas generally are slow growing, and clinical manifestations develop insidiously over many years. These include coarsening of the facial features, enlargement of the hands and feet, excessive sweating, amenorrhea, impotence, diabetes mellitus, hypertension, skin tags, and a variety of seemingly unrelated complaints, such as jaw malocclusion, carpal tunnel syndrome, colonic polyps, arthritis, and heart failure. Rarely, hyperthyroidism due to increased TSH secretion may coexist.

The diagnosis is confirmed by demonstrating excessive GH secretion. Measurement of GH in random serum samples may be inadequate, since normal values vary during the day. In patients with suggestive clinical features and normal GH levels, the glucose suppression test may be useful. Patients with acromegaly fail to suppress serum GH to <1 ng/mL following ingestion of 75 g of glucose (see Dynamic Test section). Additional confirmation is obtained by measurement of serum IGF-I and IGFBP-3 levels. Both are GH-dependent proteins, and levels usually are increased in patients with acromegaly. In patients with confirmed GH hypersecretion, pituitary imaging is conducted to identify a pituitary mass. In the absence of a pituitary tumor, an ectopic source of growth hormone releasing hormone (GHRH) must be considered. Ectopic acromegaly usually can be diagnosed by measuring serum GHRH levels in peripheral blood and by CT or MR imaging of the chest, abdomen, and/or pelvis. See the “Growth Hormone

Insulin-like Growth Factor Axis" section for further information. Treated patients should have lifelong monitoring of IGF-1 and GH suppression testing since recurrences are frequent and the recent broadening of therapeutic options with somatostatin analogues and GH antagonists gives additional opportunities for intervention. Failure to suppress GH in response to glucose and persistently high IGF-1 levels are predictors of increased morbidity and mortality and thus should be normalized if possible.

Cushing's Disease

See section on "Disorders of Adrenal Function."

Thyrotropinoma

TSH-secreting adenomas account for <1% of all pituitary tumors. They are characterized by hyperthyroidism and measurable (nonsuppressed) levels of circulating TSH. Such TSH-dependent hyperthyroidism must be distinguished from other causes of inappropriate TSH secretion (ie, thyroid hormone resistance syndromes). Co-secretion of TSH and prolactin, or GH, may occur, and alpha subunit levels may be increased. In most (88%) patients with thyrotropinoma, there is disproportionate secretion of alpha subunit and an alpha subunit/TSH ratio >1.0; in non-tumorous hyperplasia the ratio does not exceed 1.0. The diagnosis is confirmed by pituitary imaging. Although thyroid hormone resistance and TSH secreting adenomas are 2 distinct clinical entities, an association of these tumors with thyroid hormone resistance or a thyroid hormone receptor defect has been described, suggesting a possible causal relationship in some of these patients.

Gonadotropinoma

Previously, many macroadenomas now recognized by immunostaining to be gonadotropin-producing were classified as nonsecreting or chromophobe adenomas, because they usually do not produce a recognizable clinical syndrome. Thus, the classification of gonadotropinomas versus chromophobe adenomas was variable. Gonadotroph adenomas constitute 80% to 90% of all nonfunctioning pituitary adenomas using molecular, cell culture, and immunostaining techniques. Because of the minimal symptoms, gonadotropinomas are not detected early and present more commonly as macroadenomas than microadenomas, often with visual or neurological symptoms related to the mass effect. The tumors frequently produce increased serum FSH levels but only uncommonly increased serum LH concentrations. The increased biologically active levels of FSH may lead to an ovarian hyperstimulation syndrome, and elevations of LH, FSH, or both may lead to testicular enlargement or precocious puberty. About 15% of patients evidence increased alpha subunit concentrations, and both intact gonadotropin and subunit levels may increase (>30%) in response to TRH stimulation. The alpha subunit test can help differentiate primary hypogonadism from hypogonadism due to a tumor secreting nonfunctional LH and FSH.

Test Application and Interpretation

A pituitary adenoma is likely to be of gonadotroph or thyrotroph origin if the serum prolactin level is <100 ng/mL, the patient has no acromegalic features, the serum IGF-I concentration is not increased, and there is no clinical or laboratory evidence of Cushing's syndrome. In men, elevated basal levels of FSH, alpha subunit, and FSH and LH beta subunits, alone or in combination with responses of these to TRH, are strong evidence for a gonadotroph adenoma. In women, measurements of FSH are not usually helpful unless the FSH concentration is markedly increased in the presence of a normal LH value. The response of intact FSH or LH levels and of the alpha and LH beta subunits to TRH may be helpful.

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DISORDERS OF CALCIUM AND BONE METABOLISM

Background Physiology

The parathyroid glands, usually 4 in number, are small derivatives of the pharyngeal pouches located on the posterior surfaces of the bilobed thyroid gland (Figure 1). These glands are the only source of parathyroid hormone (PTH). PTH secretion is modulated by changes in concentration of free calcium in blood plasma; decreased concentrations of calcium stimulate and increased concentrations inhibit PTH secretion. PTH is an 84-amino acid protein hormone that acts 1) on the kidney tubules to increase phosphorus (P) excretion and enhance calcium reabsorption, 2) mobilize calcium from bone tissue, and 3) stimulate the 1α-hydroxylation of 25(OH) vitamin D [25(OH)D] to $1,25(OH)_2D$. This hydroxylation greatly augments the biological activity of vitamin D, and $1,25(OH)_2D$ acts on the gut to increase absorption of dietary calcium. All of these actions tend to increase the blood calcium concentration and increase the circulating blood calcium/phosphorus ratio. $1,25(OH)_2D$ also acts to potentiate bone mineralization.

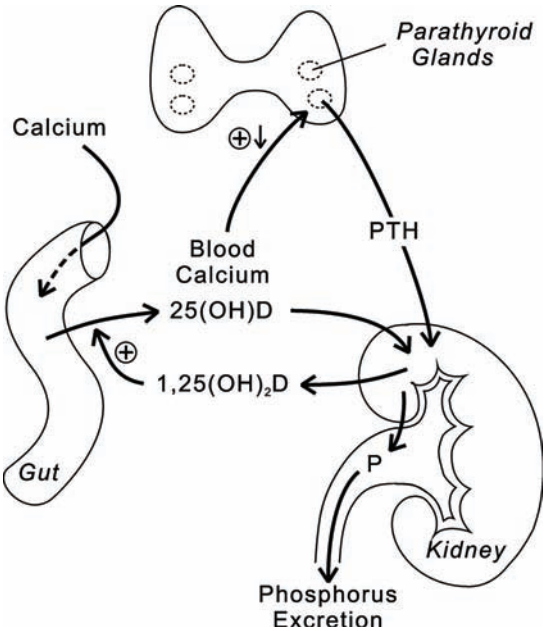

Figure 1. The control of blood calcium by parathyroid hormone (PTH) and vitamin D. See text for details.

Test Application and Interpretation

PTH is secreted predominantly as an intact molecule, but it is rapidly cleaved in peripheral tissues to amino terminus and carboxy terminus fragments. The amino terminal fragment is biologically active and has a relatively short plasma half-life. The carboxyterminal species include a 7-84 peptide and a variety of shorter fragments. They have a longer half-life but are biologically inactive. The most reliable assays for PTH measure the intact molecule.

Vitamin D is a fat-soluble vitamin stored in precursor form in skin and other tissues. Hydroxylation to 25(OH)D occurs in liver, and this form is the major circulating form of the vitamin. It serves as a precursor for 1α-hydroxylation to the active moiety [$1,25(OH)_2D$], and circulating levels of 25(OH)D serve as a marker for the adequacy of vitamin D nutrition. Vitamin D is ingested as vitamin D_2 (ergocalciferol), as D_3 (cholecalciferol), or as precursor ergosterol, which is activated to ergocalciferol in skin by ultraviolet light.

Disorders of the system occur because of congenital absence or acquired damage to the parathyroid glands (hypoparathyroidism); adenoma or hyperplasia of the glands (primary hyperparathyroidism); vitamin D deficiency; molecular defects in the PTH receptor (pseudohypoparathyroidism), the calcium sensing receptor (familial hypocalcemic hypercalciuria, neonatal severe primary hyperparathyroidism, and autosomal dominant hypocalcemia), the vitamin D receptor (vitamin D resistance), or 1α-hydroxylase (vitamin D dependence); and renal disease that limits phosphorus excretion and $1,25(OH)_2D$ production with resulting hypocalcemia and (secondary) hyperparathyroidism.

Parathyroid hormone-related protein (PTH-RP) is a protein with a 14-amino acid amino terminus analogous to the amino terminus of PTH. It binds to and activates the PTH receptor and can produce hypercalcemia. It is secreted by fetal tissues and plays a role in fetal calcium metabolism and fetal bone development. In adults, the hormone is produced in large amounts by the lactating breast and is secreted in breast milk. The physiological significance of PTH-RP in children and adults is not clear. It is, however, produced by a variety of tumor tissues and is the most common cause of the hypercalcemia of malignancy.

Calcitonin (CT) is derived from the thyroid gland C-cells, which are widely dispersed in the interfollicular tissue of the gland. CT secretion is stimulated by increased plasma calcium levels and has the capacity to inhibit the effect of PTH on bone tissue. CT levels are relatively increased in the fetus and may have a role in fetal bone anabolism. However, there is a limited role for CT in calcium or bone metabolism in children and adults; CT deficiency has no significant effect on calcium homeostasis. CT serves as a marker for medullary thyroid carcinoma, a malignancy involving the thyroid C-cells.

Laboratory Assessment of Disorders of Calcium Metabolism

The availability of sensitive and specific tests for several hormones modulating serum calcium levels has greatly improved our ability to assess disorders of calcium metabolism (Tables 1 and 2). Medullary thyroid carcinoma is discussed in the “Pheochromocytoma, Medullary Thyroid Carcinoma, and Multiple Endocrine Neoplasia” section.

Table 1. Disorders of Calcium Metabolism

Disorder	Phenotype	Etiology
Vitamin D deficiency	Hypocalcemia, defective bone mineralization, rickets, osteomalacia	Dietary deficiency
Vitamin D dependence, type I	Hereditary rickets, growth retardation, hypocalcemia	Vitamin D, 21α-hydroxylase mutation
Vitamin D dependence, type II (vitamin D resistance)	Hereditary rickets, alopecia, growth retardation, hypocalcemia	Vitamin D receptor mutations
Hypoparathyroidism	Hypocalcemia, neuromuscular irritability, cataracts, increased bone density	Autoimmune embryonic defect, surgery, radiation, PTH deficiency, other
Pseudohypoparathyroidism (PTH resistance)	Albright's hereditary osteodystrophy (variable expression), generalized hormone resistance	Gsa protein defect, *GNAS* mutations
Autosomal dominant hypocalcemia	Normal; resistance limited to PTH target tissues	Imprinting defect in *GNAS* gene
Neonatal hypocalcemia	Transient neonatal hypocalcemia	Maternal hypercalcemia
Hyperparathyroidism	Increased PTH secretion, hypercalcemia, nephrolithiasis, decreased bone mineralization	Parathyroid adenomas, MEN, *HRPT2* mutation
Tumor hypercalcemia	Hypercalcemia, PTH suppression	Tumor secretion of PTH-RP
Familial benign hypercalcemia (hypocalciuric hypercalcemia)	Osteomalacia	Calcium sensing receptor (CaSR) mutation, heterozygous
Neonatal severe hyperparathyroidism	Polyuria, hypotonia, bone demineralization, fractures	CaSR mutation heterozygous or homozygous
Secondary hyperparathyroidism (CRF)	Hypocalcemia, osteodystrophy	$1,25(OH)_2$ vitamin D deficiency, parathyroid hyperplasia
Tertiary hyperparathyroidism (ESRD)	Hypocalcemia, osteodystrophy	$1,25(OH)_2$ vitamin D deficiency, parathyroid autonomy
Medullary thyroid carcinoma	Increased calcitonin secretion, C-cell malignancy	MEN or sporadic C-cell carcinoma

PTH-RP, parathyroid hormone related protein; CRF, chronic renal failure; ESRD, end stage renal disease.

Table 2. Assessment of Disorders of Calcium-Related Hormones

Disorder	Blood Measurements							Urine Calcium
	PTH	CT	Ca	PO	25D	1,25D	PTH-RP	
Vitamin D deficiency	↑	N	N or ↓	↓	↓	↑ N or ↓	N	↓
Vitamin D dependence, type I	↑	N	↓	N or ↓	N or ↑	↓	N	↓
Vitamin D dependence, type II (vitamin D resistance)	N or ↑	N	↓	N or ↓	N	↑	N	↓
Hypoparathyroidism	N or ↓	N	↓	↑	N	N or ↓	N	↓
Pseudohypoparathyroidism (PTH resistance)	↑	N	N or ↓	N or ↑	N	N or ↓	N	↓
Autosomal dominant hypocalcemia	N or ↓	N	↓	↑ N or ↓	N	N	N	N or ↑
Neonatal hypocalcemia	N or ↓	↑	↓	↑	N	N	N or ↓	↓
Hyperparathyroidism	↑	N	↑	N or ↓	N	N or ↓	N	↑
Tumor hypercalcemia	↓	N	↑	N or ↓	N	N or ↓	N or ↑	N or ↑
Familial benign hypercalcemia (hypocalciuric hypercalcemia)	N or ↑	N	↑	↓ or N	N	N	N	↓
Neonatal severe primary hyperparathyroidism	↑	N	↑	↑ or N	N	N	N	↓
Secondary hyperparathyroidism (CRF)	↑	↑	N	↑	↓ or N	↓ or N	N	↓
Tertiary hyperparathyroidsm (ESRD)	↑↑	↑	↑	↑	↓ or N	↓	N	N/A
Medullary thyroid-carcinoma	N	↑	N	N	N	N	N	N

PTH, parathyroid hormone; CT, calcitonin; Ca, calcium; PO, phosphorus; 25D, 25-hydroxyvitamin D; 1,25 D, 1,25-dihydroxyvitamin D; PTH-RP, parathyroid hormone related protein; CRF, chronic renal failure; ESRD, end stage renal disease; N/A, not applicable.

Evaluation of Hypercalcemia

Laboratory tests are essential to evaluate patients with hypercalcemia (Table 1). The parathyroid hormone (PTH) assay plays a central role in differentiating the 2 most common causes of hypercalcemia (hyperparathyroidism and tumor-associated hypercalcemia), particularly when the PTH concentration is interpreted in relationship to the ambient serum or plasma calcium level (Figure 2). It is extremely useful in defining the most fruitful pathway for subsequent diagnostic evaluation of the hypercalcemic patient.

Parathyroid hormone-related peptide (PTH-RP), the mediator of hypercalcemia in some patients with malignancy, can be assayed directly. Vitamin D measurements are also helpful in identifying patients with exogenous vitamin D intoxication and excessive endogenous vitamin D activation (eg, sarcoidosis and lymphoma).

Other causes of hypercalcemia include hyperthyroidism, immobilization, thiazide diuretics, vitamin D intoxication, vitamin A intoxication, the milk-alkali syndrome, and lithium therapy. Other than lithium therapy, which causes increased PTH secretion through a shift in the dose response curve relating serum calcium to PTH secretion, these causes of hypercalcemia are associated with suppression of serum PTH levels.

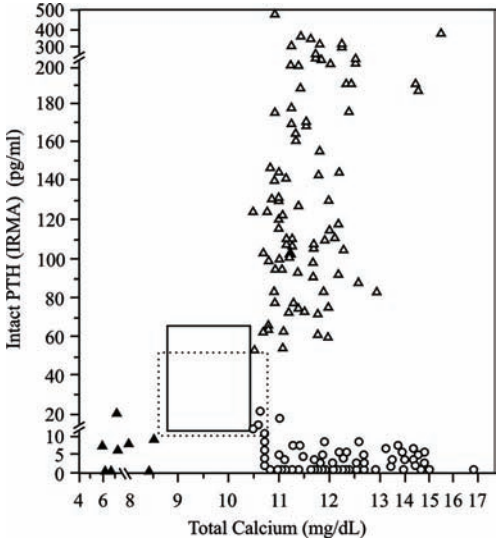

Figure 2. Relationship of serum calcium and intact PTH (IRMA or ICMA) in various disease states. The hatched square indicates normal pediatric range. ▲, hypoparathyroidism; △, primary hyperparathyroidism; O, hypercalcemia of malignancy.

Familial hypocalciuric hypercalcemia (FHH), a familial benign hypercalcemia, is an autosomal dominant trait. The hypercalcemia is asymptomatic in most subjects and most prominent in the first decade of life. The hypocalciuria is due to increased renal tubular calcium reabsorption. Serum PTH levels are in the normal range but inappropriate for the serum calcium level. The defect is due to a loss of function mutation of the gene on chromosome 3 for the calcium-sensing receptor that is expressed in kidney, parathyroid cell, and other tissues. The heterozygous state causes FHH (as a dominant negative allele), while the homozygous state produces severe neonatal hypercalcemia. Diagnosis requires demonstrating hypocalciuria and reduced renal calcium clearance in the context of a family history of these disorders. Mutational analysis of the calcium sensing receptor confirms the diagnosis in those with a family history and is necessary for the diagnosis in patients without a family history.

Evaluation of Hypocalcemia

The causes of hypocalcemia are listed in Table 1. Hypoparathyroidism can be due to damage to the parathyroid glands (surgery, infiltrative disease, irradiation, chemotherapeutic drugs, sepsis), to autoimmune disorders, or to molecular genetic defects. The latter involve autosomal defects linked to the PTH gene on chromosome 11 as well as the yet to be defined X-linked variety. Activating mutations in the calcium sensing receptor causing autosomal dominant hypocalcemia (ADH) are also causative.

Test Application and Interpretation

Pseudohypoparathyroidism, or PTH resistance, is due to a defect in the renal PTH receptor-adenylate cyclase complex (type I) or a more distal defect blocking the renal phosphaturic response to cAMP (type II). Vitamin D deficiency, dependence, and resistance often are associated with rickets or osteomalacia. Type I vitamin D dependence is due to a functional defect in the 25(OH) vitamin D, 1α-hydroxylase enzyme in the kidney, impairing activation of 25(OH) vitamin D to $1,25(OH)_2$ vitamin D. Type II dependence is due to a defective vitamin D receptor.

Transient neonatal hypocalcemia is due to chronic fetal hypercalcemia associated with maternal hyperparathyroidism. Recovery of parathyroid gland function, which is suppressed in utero, is delayed, and there is a period of relative hypoparathyroidism lasting several days to 2 to 4 weeks in the early neonatal period.

Vitamin D Deficiency

Vitamin D is essential for optimal gastrointestinal absorption of calcium and maintenance of bone mineral density (BMD). Deficiency is relatively common, especially in the elderly. Daily requirements vary with age, calcium intake, and sunlight exposure. An intake of 400 IU/d is recommended for infants and children and for adults with minimal sunlight exposure. In the elderly, efficacy of UV light-stimulated synthesis of vitamin D in the skin is reduced; thus, a minimal daily intake of 600 IU is recommended. To prevent osteoporotic fractures in elderly individuals, 800 IU/d of vitamin D and 1200 mg/d of calcium are recommended. The recommended upper limit of intake is 2,000 IU/d.

Endogenous vitamin D is D_3 (cholecalciferol), whereas supplemental vitamin D in the United States is primarily D_2 (ergocalciferol). The adequacy of vitamin D levels is assessed by measuring serum levels of 25(OH)D; however, some immunoassays underestimate D_2 and thus total circulating 25(OH)D. The tandem mass spectrometry method, on the other hand, accurately detects and quantifies both D_2 and D_3 and thus is the method of choice when monitoring patients taking vitamin D supplements.

Figure 3 shows the relationship of serum 25(OH)D levels vs PTH concentrations in apparently healthy 18 to 65 year-old individuals. PTH concentrations increase perceptibly when 25(OH)D levels fall below 30 ng/mL and are clearly increased at 25(OH)D concentrations of 10 ng/mL in agreement with earlier studies. BMD of the femoral neck also has been shown to be relatively decreased at serum 25(OH)D levels <30 ng/mL, and secondary hypoparathyroidism with increased bone turnover is associated with serum 25(OH)D concentrations <15 ng/mL. Optimal serum 25(OH)D concentrations appear to fall in the 30 to 80 ng/mL range, particularly in elderly people.

Vitamin D Metabolism in Renal Failure

Bone and mineral metabolism is gradually altered as renal failure progresses in chronic kidney disease (CKD). By the time of progression to end stage renal disease (ESRD), all subjects with renal failure are affected by metabolic bone

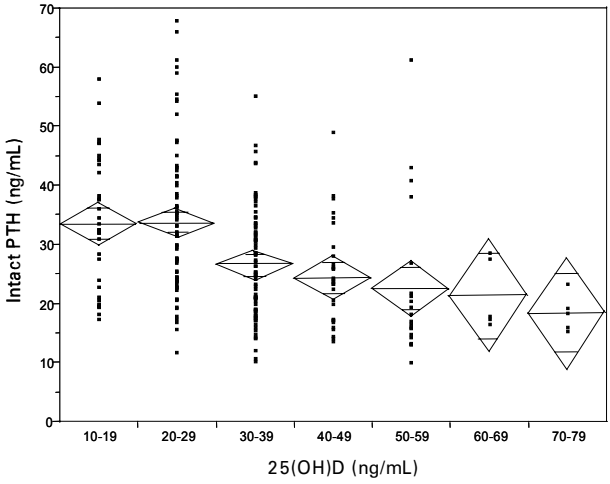

Figure 3. 25-Hydroxyvitamin D vs. PTH levels in 282 apparently healthy individuals with normal serum calcium (8.8-10.1 mg/dL) and serum creatinine (0.5-1.4 mg/dL) levels.

disease and are at increased fracture risk. A host of factors contribute to this including 1) the loss of 1α-hydroxylation of 25(OH) vitamin D in the kidneys, 2) the inability to excrete phosphorus, 3) hypocalcemia, and 4) increased resistance to the action of PTH. These factors lead to a gradual increase in PTH levels.

The above usually occurs in a population already prone to increased risk for 25-hydroxyvitamin D deficiency. A host of factors in subjects with advanced renal failure or on dialysis contribute to this increased risk such as 1) relatively decreased exposure to sunlight, 2) decreased efficiency in skin synthesis of vitamin D_3, 3) diabetes, 4) renal wasting of vitamin D and vitamin D binding protein in subjects with the nephrotic syndrome, and 5) loss of vitamin D in dialysate of subjects on peritoneal dialysis. The net effect is a decreased substrate for 1α-hydroxylation and a further decrease in $1,25(OH)_2$ vitamin D levels.

The initial rise in PTH levels leads to a state of secondary hyperparathyroidism in which a normal calcium level is maintained at the expense of a high serum PTH level (Table 1). This is seen as early as stage 3 CKD (Table 3). If this state of secondary hyperparathyroidism is left untreated, the constant stimuli for increased PTH synthesis leads to parathyroid hyperplasia with lack of sensing of ambient ionized calcium and eventual autonomy of the parathyroid glands. On occasion, neoplasia with adenomatous transformation in one or more of the glands is observed. This leads to a state of tertiary hyperparathyroidism characterized by high PTH levels and hypercalcemia. In both secondary and tertiary hyperparathyroidism, calcium is mobilized from the skeleton leading to renal osteodystrophy.

Table 3. Frequency of Measurement of PTH and Calcium/Phosphorus by Stage of CKD

CKD Stage	GFR Range mL/Min/1.73 m^2	Measurement of PTH	Measurement of Calcium/Phosphorus
3	30-59	Every 12 months	Every 12 months
4	15-29	Every 3 months	Every 3 months
5	<15 or dialysis	Every 3 months	Every month

To prevent this sequence of events, it is recommended that all the causes of renal bone disease be treated as early as the disease is identified. The National Kidney Foundation for Bone Metabolism and Disease in Chronic Kidney Disease (K/DOQI Clinical Practice Guidelines) recommends the monitoring of serum calcium, phosphorus, and PTH levels, as indicated in Table 4, and maintaining PTH levels within the parameters provided in Table 3. This can be achieved through a variety of measures including control of phosphorus levels through phosphate binders, correction of 25-hydroxyvitamin D deficiency (<20 ng/dL) and insufficiency (<32 ng/dL) through pharmacologic doses of D_2 or D_3. After vitamin D sufficiency is achieved, it is appropriate to treat with active vitamin D metabolites such as calcitriol (Table 5).

Metabolic Bone Disease

Bone is an unusual tissue because it is largely mineral and undergoes an active process of remodeling. Bone remodeling occurs in 2 stages, resorption followed by replacement or formation. These processes are mediated by different bone cell types, osteoclasts and osteoblasts, respectively; however, the processes are coupled so that, usually, no net bone is lost. During resorption, the matrix or scaffolding of bone, which is composed of mineralized deposits and proteins, is solubilized and digested by acid and enzymes produced by the osteoclasts. Tartrate resistant acid phosphatase (TRAP) is one such enzyme and is a marker of osteoclastic activity. The matrix protein degradation products (cross-linked N-telopeptide [NTx], C-telopeptide (CTx), deoxypyridinoline [DPYD], pyridinoline [PYD], and hydroxyproline from bone collagen) are released into the extracellular environment and are excreted in the urine. Measurement of these breakdown products allows assessment of the rate of bone resorption.

Osteoblasts, unlike osteoclasts, synthesize collagen and other matrix proteins and are responsible for deposition of the matrix, which is then mineralized. Collagen is synthesized as a large precursor procollagen protein, which is

Table 4. Target Range of Intact Plasma PTH by Stage of CKD

CKD Stage	GFR Range mL/min/1.73m^2	Target "intact" PTH pg/mL (pmol/L)
3	30-59	35-70 (3.85-7.7)*
4	15-29	70-110 (7.7-12.1)*
5	<15 or dialysis	150-300 (16.5-33.0)†

*Opinion-based.
†Evidence-based.

Table 5. Recommended Supplementation for Vitamin D Deficiency/Insufficiency in Patients with CKD Stages 3 and 4

Serum 25(OH)D ng/mL (nmol/L)	Definition	Ergocalciferol (Vitamin D_2) Dose	Duration Months	Comment
<5 (12)	Severe vitamin D deficiency	50,000 IU/wk orally x 12 wks, then monthly	6	Measure 25(OH)D levels after 6 months
5-15 (12-37)	Mild vitamin D deficiency	50,000 IU as single I.M. dose x 4 weeks, then monthly	6	Assure patient adherence; measure 25(OH)D at 6 months
16-30 (40-75)	Vitamin D insufficiency	50,000 IU/month orally	6	

processed prior to its incorporation into bone matrix. Osteoblasts also synthesize noncollagenous matrix proteins such as osteocalcin (OC) and enzymes such as bone specific alkaline phosphatase (BSAP). Circulating levels of OC and BSAP reflect osteoblast activity and bone formation. Table 6 summarizes the biochemical markers of bone turnover. Tables 7 and 8 list the methodologies and normal values for the bone resorption and formation markers. Reference range curves for normal values in children from 2 months to 18 years have been recently published (Ref 16).

Clinical Application of Bone Markers

Biochemical markers of bone metabolism are useful diagnostic tools because they reflect acute changes in bone-remodeling activity. They complement measurement of bone density, which measures the cumulative balance of bone resorption/formation over a longer time interval.

Diagnosis of Bone Disorders

Table 9 outlines the use of bone metabolism markers in the diagnosis of a number of bone-related disorders. Levels of all markers are clearly elevated in Paget's disease and in primary hyperparathyroidism. In addition to their usefulness in diagnosing these disorders, DPYD and NTx are also useful for monitoring the activity of these disease processes and their response to therapy.

Table 6. Biochemical Markers of Bone Turnover

Biochemical Marker	Abbreviation
Resorption	
Deoxypyridinoline	DPYD
Pyridinoline	PYD
Cross-linked N-telopeptide	NTx
Collagen type I C-telopeptide	CTx
Tartrate resistant acid phosphatase	TRAP
Formation	
Osteocalcin	OC
Bone specific alkaline phosphatase	BSAP

Table 7. Bone Resorption Markers

	NTx	CTx	Free DPYD	PYD Collagen Cross-links		Hydroxyproline	TRAP
				DPYD	PYD		
Sample Type	Urine (2nd AM void)	Serum	Urine (2nd AM void)	Urine (2nd AM void, 2-h, 24-h)	Urine (2nd AM void, 2-h, 24- h)	Urine (2nd AM void or 24-h)	Serum
Method	ICMA	ECL	ICMA	HPLC	HPLC	Colorimetric	Enzymatic
Analytical sensitivity	10 nmol BCE/L	30 pg/mL	20 nmol/L	13 pmol/mL	20 pmol/mL	2 µg/mL	1.2 U/L
Reference ranges	nmol BCE/mmol creat	pg/mL	nmol/mmol creat	nmol/mmol creat	nmol/mmol creat	mg/24-h	U/L
Men							
U, 2nd AM void, 2-h			2.1-8.1	6.2-25.6	22.7-65.2		
18-29 y	12-99						
30-59 y	9-60						
U, 24-h	5-87			3.7-18.7	20.0-60.8	9-73	
S							3.0-6.0
18-29 y		87-1200					
30-39 y		70-780					
40-49 y		60-700					
50-68 y		87-345					
Women, premenopausal							
U, 2nd AM void, 2-h	4-64		3.3-13.5	5.7-23.3	25.4-82.8		
U, 24-h	5-79			4.4-21.1	22.1-88.9	7-49	
S							3.0-6.0
18-29 y		64-640					
30-39 y		60-650					
40-49 y		40-465					
Children			See individual assays in Alphabetical Test Section				
Clinical Use	Monitor therapy (osteoporosis & Paget's disease)		Assess bone turnover	Assess bone turnover	Assess bone turnover	Assess bone turnover	Assess bone turnover

NTx, N-telopeptide; CTx, C-telopeptide; DPYD, deoxypyridinoline; PYD, pyridinoline; TRAP, tartrate resistant acid phosphatase; BCE, bone collagen equivalent; ICMA, immunochemiluminometric assay; ECL, electrochemiluminescence assay; immunoassay; HPLC, high pressure liquid chromatography; U, urine; S, serum.

Table 8. Bone Formation Markers

	BSAP*		Osteocalcin†	
Sample type	Serum		Serum	
Method	IRMA		IRMA	
Analytical Sensitivity	2.0 µg/L		2.0 ng/mL	
Reference ranges	µg/L	µg/L		ng/mL
Adults	Men	Women	Men	11.3-35.4
18-29 y	8.4-29.3	4.7-17.8	Women	7.2-27.9
30-39 y	7.7-21.3	5.3-19.5		
40-49 y	7.0-18.3	5.0-18.8		
50-68 y	7.6-14.9			
50-76 y		5.6-29.0		
Children*	Male	Female	Children†	
2-24 mo	25.4-124.0	25.4-124.0	6-9 y	40-2-108.0
6-9 y	41.0-134.6	41.0-134.6	10-13.9 y	35.8-165.5
10-13 y	43.8-177.4	24.2-154.2	14-17.9 y	
14-17 y	13.7-128.0	10.5-75.2	Males	27.8-194.1
			Females	16.3-68.7
Clinical use	Monitor therapy in osteoporosis and Paget's disease		Assess bone turnover	

BSAP, bone specific alkaline phosphatase, BAP, skeletal alkaline phosphatase; osteocalcin, bone gla protein, BGP.
*Pediatric data from *Int J Biol Markers*. 1996;11:159-164.
†Pediatric data from Quest Diagnostics Nichols Institute Clinical Correlations Department.

Because of the coupling of bone resorption and formation, utilizing results from a single biochemical marker of bone turnover, either formation or resorption, is not as informative as the use of markers for both processes. It is likely that the relative values or ratios of formation and resorption markers will prove to be more informative in evaluating bone disorders and their treatment than the use of single markers or absolute levels.

Table 9. Effect of Disease State on Biochemical Markers of Bone Turnover

	Resorption Markers			Formation Markers	
Disease	DPYD	PYD	NTx	OC	BSAP
Osteoporosis	↑↑	↑	↑	Normal	Normal
Paget's Disease	↑↑↑	↑↑↑	↑↑↑	↑↑	↑↑↑
Hyperparathyroidism	↑↑	↑↑	↑↑	↑↑	↑↑

↑, marginally elevated relative to normal; ↑↑, elevated relative to normal; ↑↑↑, highly elevated relative to normal.

Test Application and Interpretation

Diagnosis of Osteoporosis

The current practice for diagnosis of osteoporosis is bone densitometry. Since bone density can decline at a rate of 1% to 3% per year, bone densitometry is a good method of detecting established osteoporosis. It has limited usefulness, however, for immediate follow-up of treatment of osteoporotic patients because subtle changes in bone density cannot be detected. Measurements of biochemical markers of bone formation and resorption (Table 6), unlike bone densitometry, are particularly well suited for monitoring the efficacy of treatment. These markers will change rapidly if treatment, such as estrogen therapy, is effective.

In osteoporosis, the rate of bone resorption may be only modestly increased after menopause, as indicated by the overlap in the reference ranges for these markers in premenopausal and postmenopausal women. Thus, the usefulness of these markers in diagnosis of primary osteoporosis is limited. The markers are most useful in cases of high bone turnover. Additionally, in the majority of cases, the biochemical markers will be most useful in evaluation of the efficacy of treatment. Patients with high levels of bone turnover markers may be the most suitable for treatment with antiresorptive agents. In this case, following the bone turnover markers at a later time will be especially useful in determining efficacy of treatment.

Efficacy of Therapy

Measurement of the levels of biochemical markers can be used to determine whether a drug therapy is effective within 2 to 6 months. A decrease in the levels of the markers of >25% indicates effective intervention.

Follow-up to Therapy

A similar approach can be used to determine the appropriate dose of drug. By following the changes in biochemical bone markers, the level of drug administered can be fine-tuned to achieve the desired level of inhibition of bone resorption to maintain an acceptable rate of bone remodeling.

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DISORDERS OF CARBOHYDRATE METABOLISM

Background Physiology

In the fasting or post absorptive state, blood glucose concentration is maintained in a narrow range (about 70 to 100 mg/dL) by a balance of hormonal actions (Figure 1). Principal among these is the level of circulating bioactive insulin. In response to rising glucose levels, pancreatic insulin secretion is stimulated, glucose uptake by liver and peripheral tissues is increased, glycogen synthesis is augmented, and hepatic glucose output is suppressed. The net result is a reduction in blood glucose concentration with an increase in carbohydrate stores (Figure 1). Hypoglycemia results in reduced insulin secretion and stimulation of glucagon, growth hormone, cortisol, and, when severe, catecholamine secretion. These counter-regulatory hormones initiate glycogenolysis, gluconeogenesis, and increased hepatic glucose output to raise blood glucose at the expense of carbohydrate and substrate stores.

Hyperglycemic Disorders

The commonest disorders of carbohydrate metabolism are the several types of diabetes mellitus involving decreased insulin secretion or action.Type 1 diabetes mellitus, also referred to as insulin dependent diabetes mellitus (IDDM), is characterized by persistent hyperglycemia, usually with ketosis and low levels of

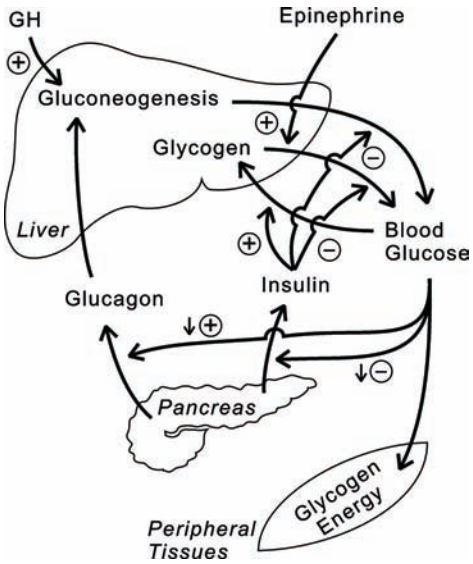

Figure 1. Features of carbohydrate metabolism. See text for details.

circulating insulin, C-peptide, and proinsulin. Type 1 diabetes is due to chronic autoimmune pancreatic islitis with progressive destruction of the insulin-secreting beta cells of the pancreatic islets. Insulin and islet cell autoantibodies are present in most patients with type I diabetes at diagnosis and the disease usually is manifest during the first 2 decades of life. Other autoantibodies and autoimmune diseases, especially autoimmune thyroid disease, are present in 10% to 30% of the patients with type I diabetes.

Type 2 diabetes mellitus, sometimes referred to as adult onset disease or non-insulin dependent diabetes mellitus (NIDDM), represents a more heterogeneous group of disorders associated with insulin resistance and variable degrees of hyperinsulinemia and hyperglycemia without ketosis. It is the predominant form of the disease worldwide. Autoimmunity may contribute to the pathogenesis in type 2 disease since 7% to 10% of adult diabetes mellitus is associated with positive autoimmune markers.

Type 1 Diabetes

Research during the past two decades has identified circulating autoantibodies directed against a variety of pancreatic islet cell antigens (ICA) in patients with IDDM and their first-degree relatives. These antigens are summarized in Table 1.

The pancreatic beta cell autoimmune destructive process occurs over an extended period of time during which the patient is asymptomatic and euglycemic. Clinical glucose intolerance develops as the residual islet beta cell mass is progressively reduced to 10% or less. Continuing destruction leads to overt diabetes mellitus. The prolonged prediabetic stage of the disease makes it possible to utilize immunologic markers to identify individuals at risk and estimate the severity of the autoimmune process. The presence of autoantibodies directed to 2 or more characteristic autoantigens indicates a more severe autoimmune process and is associated with a higher risk for progression to overt disease.

Table 1. Antigens for Which Autoantibodies Have Been Detected in Patients with IDDM

Insulin
Glutamic acid decarboxylase (GAD)
IA-2 (ICA 512, 40 kd)*
Phogrin (IA-2b, 37 kd)*
GM2-1 ganglioside
52 kd ICA $\alpha^{\dagger}$
69 kd ICA $\alpha^{\dagger}$
Carboxypeptidase H
Pancreative sulfatide
Glucose transporter

*A member of the protein tyrosine phosphatase (PTP) family.
†Protein with unknown function.

Test Application and Interpretation

Cytoplasmic islet cell autoantibody measured by immunofluorescence using histologic sections of human or monkey pancreas is semi-quantitative and difficult to standardize. Direct methods using radioassay or ELISA formats have been developed utilizing purified or recombinant protein antigens. These include insulin, GAD, and ICA-512 (IA-2) antigens. These assays, used in combination, provide high sensitivity and reliability for the diagnosis of pancreatic islet beta cell autoimmune disease. The assays allow identification of at-risk individuals in families with type 1 diabetes, the identification of late onset type 1 disease in adult patients, and, with studies of first phase insulin release in prediabetic individuals, allow monitoring of progress of the autoimmune state. Diagnosis of the prediabetic phase allows patient monitoring and early institution of insulin therapy after documentation of hyperglycemia prior to metabolic decompensation (eg, ketoacidosis). Diagnosis of the prediabetic state will be essential to early institution of immunomodulatory therapies as these become available.

Verge et al (*Diabetes.* 1996;45:926, Ref. 2) studied 882 first degree relatives (median age 13 years) of patients with type 1 diabetes. Fifty of the relatives developed diabetes during a median follow-up of 2 years (0.1-11.3 years). Among the 50, 98% had autoantibodies to 1 or more antigens (GAD, ICA 512, insulin), and 80% had 2 or more autoantibodies. None of 200 control subjects expressed more than a single autoantibody.

Table 2 summarizes the risk of IDDM in the 882 relatives according to the number of antibodies detected.

Kulmala et al (*J Clin Invest.* 1998;101:327, Ref. 3) assessed autoantibody prevalence (ICAA, GAD, IA-2, insulin) in an unselected population of 755 siblings of children with type 1 disease. The risks for disease in siblings with 4, 3, 2, 1, or no antibodies were 40%, 70%, 25%, 2%, and 0.8%, respectively. Sensitivity of combined GAD and IA-2 was 81% and the positive predictive value was 41%. Bilbao et al (*Hormone Res.* 2000;54:181, Ref. 5) reported that of 448 recent onset type 1 patients, 90% were positive for at least 1 antibody and 68% presented with 2 or more autoantibodies.

Caveats of autoantibody testing include lack of high risk of diabetes for most individuals expressing only a single autoantibody unless there is loss of first phase insulin secretion (<1st percentile of normals) on intravenous glucose tolerance

Table 2. Risk of Development of Overt IDDM in First Degree Relatives of IDDM Patients Relative to the Presence of Insulin, GAD and/or ICA512 Autoantibodies*

Number of Autoantibodies	Risk (%) of IDDM within		
	3 Years	5 Years	10 Years
0	<1	<1	<1
1	8	15	23
2	30	43	72
3	49	>95	

*Verge et al. *Diabetes.* 1996;45:926. (Ref. 2)

testing. This is particularly true for individuals with the protective HLA alleles DQA1*0102, DQB1*0602, relatives, and autoimmune polyglandular syndrome II (APS-II) patients with high titer islet cell immunofluorescence but only GAD autoantibodies. Insulin autoantibodies are predominantly, but not exclusively, found in children developing type 1 diabetes. Subcutaneous insulin therapy, even with human insulin, induces insulin antibodies and thus positive results are not reliable if an individual has had more than approximately 1 week of insulin therapy. Of note, GAD and ICA 512 often remain positive for up to 2 years after the development of symptomatic disease. Finally, a small subset of individuals developing type 1 diabetes lack GAD, insulin, and ICA 512 autoantibodies.

Risk factors for autoimmune type 1 disease are summarized in Table 3.

Table 3. Diagnosis of Increased Diabetes Risk

Transient hyperglycemia (<10% with ≥1 Ab)	≥1 Ab: high risk insulin dependence
Gestational diabetes (<10% with ≥1 Ab)	≥1 Ab: high risk insulin dependence
First degree relatives of patient with type 1 diabetes:	
Offspring of father with type 1	≥2 Ab: high risk; 0 Ab: risk <1/300
Offspring of mother with type 1	≥2 Ab: high risk; 0 Ab: risk <1/300
Sibling	≥2 Ab: high risk; 0 Ab: risk <1/300
Parent	≥2 Ab: high risk; 0 Ab: risk <1/300
Monozygotic twin	≥1 Ab: high risk; 0 Ab: risk > general population
Potential renal transplant donor	Increased risk with antibody as indicated for relatives (see above); intravenous GTT and OGTT useful
DQA1*0102/DQB1*0602 "protected" relative	1 Ab: low risk; ≥2 Ab: unknown risk; may be low
APS II patient Graves disease Addison's disease Myasthenia gravis Celiac disease	High titer single antibody: only modestly increased risk
APS I patient	High titer single antibody: only modestly increased risk
General population (<1/300 express >2 Ab)	
High/moderate risk HLA alleles	≥2 Ab: similar to sibling risk
DQA1*0102/DQB1*0602	0 Ab: risk <1/300; ≥2 Ab: risk currently unknown

Ab, antibody; APS, autoimmune polyglandular syndrome.

Maturity Onset Diabetes of the Young (MODY)

Maturity onset diabetes of the young (MODY) is a subclass of type 2 disease recognized in children, adolescents, and young adults and usually transmitted as an autosomal dominant trait. The patients may or may not be obese. Six mutated genes have been described in MODY patients (Table 4).

All of the nuclear factors and IPF-1 function as transcription factors regulating insulin biosynthesis. Heterozygous mutations cause diabetes related to beta cell dysfunction. Homozygous mutation of IPF-1 has been shown to produce islet cell aplasia. Other defects limiting insulin secretion may be involved in MODY presenting with obesity and with or without ketoacidosis.

Type 2 Diabetes

The pathogenesis of type 2 diabetes involves interaction of genetic and environmental factors. Cardinal features include insulin resistance, defective insulin secretion in response to glucose stimulation, and increased hepatic glucose production. Factors predisposing to insulin resistance include a familial history of diabetes, increased body fat content (body mass index, BMI), particularly central (intra-abdominal) adiposity, and relative inactivity. Insulin resistance leads progressively to relative insulin deficiency and increasing fasting plasma glucose. Other associated features have been described as the metabolic syndrome, which the WHO has defined as a waist circumference/hip circumference ratio >0.90, BMI >30, increased fasting plasma glucose (>110 mg/dL), dyslipidemia (serum triglycerides >150 mg/dL and HDL cholesterol <35 mg/dL), and blood pressure >140/90 mm Hg. The syndrome in middle-aged men is associated with a 2- to 3-fold increase in cardiovascular disease mortality and a 2-fold increase in overall mortality (Ref 8).

Type 2 diabetes has become a significant health problem in childhood and adolescence now accounting for 8% to 45% of new pediatric diabetic patients. The increasing prevalence has paralleled the increase in BMI in children during the past 20-30 years, and the pathogenesis appears to mirror that in adult patients.

Table 4. Mutated Genes in MODY Patients

MODY Type	Mutated Gene	Prevalence
MODY 1	HNF 4α	Rare
MODY 2	Glucokinase	Common
MODY 3	HNF 1α	Common
MODY 4	IPF-1	Rare
MODY 5	HNF 1β	Rare
MODY 6	Neuro D1/BETA 2	Rare

HNF, hepatocyte nuclear factor; IPF, islet-promoting factor also known as IDX-1, a homeobox gene; Neuro D1/BETA 2, neurogenic differentiation 1/beta cell E-box transactivator.

Risk factors include a positive family history, obesity, polycystic ovarian syndrome (PCOS), acanthosis nigricans, ethnicity (higher prevalence in minority populations), and intrauterine programming with increasing insulin resistance leading to relative insulin deficiency and overt diabetes. Although the absence of diabetes autoimmune markers is presently a prerequisite for the diagnosis of type 2 diabetes in pediatric patients, the prevalence of such markers appears to be similar to the 7% to 10% prevalence in adult type 2 patients.

Female patients with variable degrees of insulin resistance associated with insulin autoantibody, variable carbohydrate intolerance, acanthosis nigricans, and PCOS have been referred to as type B insulin resistant. Males also manifest the disease but without gonadal dysfunction. Diabetes also may be due to a variety of insulin receptor mutations. Females with insulin resistance type A associated with inactivating mutations in the insulin receptor gene also display acanthosis nigricans and PCOD; these features are believed to be secondary to the hyperinsulinemia, but the mechanism is not yet understood. The Rabson-Mendenhall syndrome is manifest by leprechaun-like features, acanthosis nigricans, and severe insulin resistance due to insulin receptor mutation. Heterozygous mutations in the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) are associated with severe insulin resistance and early onset type 2 diabetes. Additionally, a common polymorphism in PPARγ (Pro12Ala) predisposes to insulin resistance with an increased risk of type 2 diabetes. Impaired islet beta cell function also has been associated with a maternally transmitted mutation in mitochondrial transfer RNA with variable phenotypic expression resembling type 1 or type 2 diabetes. These disorders are summarized in Table 5, which details the blood tests available for assessing patients with hyperglycemia.

Diagnosis of Diabetes Mellitus

The diagnostic criteria for impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus are shown in Table 6. Venous blood from an antecubital vein is preferable to capillary blood samples for glucose tolerance testing. Plasma or serum can be used for glucose measurements. Standard tests are done in the morning after 12 to 14 hours of fasting. No formal dietary preparation is necessary unless the patient has been on a restricted diet.

Criteria for testing for diabetes mellitus in pediatric and asymptomatic adult subjects are summarized in Tables 7 and 8.

Preventing and Managing Complications

The major complications of diabetes include dyslipidemia, cardiovascular disease, ophthalmopathy, nephropathy, and neuropathy. Laboratory tests are useful tools for prevention and management of these complications.

The American Diabetes Association recommends the following tests be considered for initial laboratory evaluation of a patient diagnosed with diabetes (Ref. 15):

- Hemoglobin A_{1c}

Table 5. Assessment of Hyperglycemia

Disorder	Blood Measurements							Other
	Insulin	Proinsulin	C-Peptide	Islet Cell Antibodies	Insulin Antibody*	Insulin Receptor Antibody	Ketosis	
Diabetes mellitus								
Type 1	↓	↓	↓	↑	↑	No	Yes	Other tissue autoantibodies present; usually childhood onset
Insulin resistance, type B	↑	↑	↑	No	No	Yes	No	Insulin receptor autoantibodies; acanthosis nigricans; other autoimmune disease
Type 2	N or ↑	↑	N or ↑	7%[2]	N or↑[†]	No	No	Often obese
Gestational diabetes	N or ↑	↑	N or ↑	7%[2]	±	No	No	Transient diabetic state; risk of permanent disease
Monogenic forms of diabetes								
Permanent neonatal diabetes	↓	↓	↓	No	No	No	Yes	Activating mutation of ATP-sensitive potassium-channel subunit Kir6.2
MODY	N or ↑	N	N or ↑	No	No	No	No	Usual dominant inheritance
Insulin resistance, type A	N or ↑	N or ↑	N or ↑	No	No	No	No	Acanthosis nigricans; PCOD in females; insulin receptor mutation
Rabson-Mendenhall syndrome	↑	↑	↑	No	No	Yes	No	Insulin receptor mutation, leprechaun-like features, precocious puberty
Leprechaunism	↑	↑	↑	No	No	No	No	Insulin receptor mutation, abnormal facies, growth retardation, early death
Mutant insulin	N or ↑	↑	↓	No	No	No	No	Autosomal dominant trait; insulin responsive
Lipoatrophic diabetes	↑	↑	↑	No	No	No	No	Several genetic forms
Mitochondrial gene mutations	V	V	V	No	No	No	No	Maternal transmission
PPARγ mutation	↑	↑	↑	No	No	No	No	Early onset type 2 phenotype, severe insulin resistance

MODY, maturity onset diabetes of the young; PCOD, polycystic ovarian syndrome; V, variable: type 1 or type 2 phenotype.

*Prior to any insulin therapy.

[†]Increased in 7% of late onset type 1 patients.

Table 6. Diagnostic Thresholds for Diabetes and Lesser Degrees of Impaired Glucose Regulation*

	Fasting Plasma Glucose mg/dL	2-h Plasma Glucose mg/dL
Normal	<100	<140
IFG†	100-125	Not applicable
IGT†	Not applicable	140-199
Diabetes‡	≥126	≥200

**Diabetes Care.* 2006;29(Suppl 1):S4-S48.
†When both tests are performed, IFG or IGT should be diagnosed only if diabetes is not diagnosed by the other test.
‡A diagnosis of diabetes needs to be confirmed on a separate day.

Table 7. Pediatric Testing Criteria for Type 2 Diabetes Mellitus*

Overweight…
- BMI >85th percentile for age and sex
- Weight >85th percentile for height
- Weight >120% of ideal for height

…plus any 2 of the following:
- Family history of type 2 diabetes in first or second degree relative
- Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)
- Signs of insulin resistance or associated conditions (acanthosis nigricans, hypertension, dyslipidemia, PCOS)
- Maternal history of diabetes or GDM

BMI, body mass index; PCOS, polycystic ovary syndrome; GDM, gestation diabetes mellitus.
*Consider testing at 10 years of age or at onset of puberty if puberty occurs <10 years; frequency: every 2 years; FPG test preferred.
From *Diabetes Care.* 2006;29(Suppl 1):S4-S48.

Table 8. Criteria for Diabetes Testing in Asymptomatic Adults

- Age ≥45 years, at 3-year testing interval
- Any age if BMI ≥25 kg/m^2 plus one or more of the following:
  - Inactivity
  - First degree relative with diabetes
  - Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)
  - Delivered baby >9 lb or diagnosis of GDM
  - Hypertensive (≥140/90 mm Hg)
  - HDL cholesterol <35 mg/dL and/or triglyceride >250 mg/dL
  - PCOS diagnosis
  - IGT or IFG on previous test
  - Features of insulin resistance (eg, acanthosis nigricans)
  - History of vascular disease

Diabetes Care. 2006;29(Suppl 1):S4-S48.
BMI, body mass index; GDM, gestational diabetes mellitus; PCOS, polycystic ovary syndrome; IGT, impaired glucose tolerance; IFG, impaired fasting glucose.

Test Application and Interpretation

- Total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol
- Liver function tests; if abnormal, add tests for fatty liver or hepatitis
- Microalbuminuria
  - In patients with type 1 diabetes for ≥5 years, perhaps earlier in pubertal children
  - In all patients with type 2 diabetes
- Creatinine and estimated glomerular filtration rate (eGFR)
- TSH in all patients with type 1 diabetes and, when clinically indicated, in patients with type 2
- Electrocardiogram in adults, if clinically indicated
- Urinalysis, including ketones, protein, and sediment examination

Laboratory testing recommendations (Ref. 15) for on-going patient management follow. Table 9 summarizes the clinically significant concentrations for each test.

- Self-monitoring of blood glucose (≥3/day for patients receiving multiple insulin injections)
  - Adults: pre- and postprandial
  - Children: preprandial and postprandial if disparity between A_{1c} and preprandial levels
- Hemoglobin A_{1c} determination at least 2 times per year in patients who are meeting treatment goals and have stable glycemic control and quarterly in patients whose therapy has changed or treatment goals are not being met
- Lipids (LDL, triglycerides, HDL)
  - Adults: annually
  - Prepubertal children: not recommended unless there is a family history of hypercholesterolemia or cardiovascular event before age 55 or family history is unknown
  - Pubertal children (>12 years of age): every 5 years if levels indicate acceptable risk
  - Children, all ages: annually if lipids are abnormal
- Microalbumin
  - Adults: annually in those with type 1 diabetes for ≥5 years and in all with type 2 diabetes (starting at diagnosis and during pregnancy)
  - Children: annually in those with diabetes for ≥5 years (beginning at 10 years of age)
- Creatinine and eGFR, at least annually in all adult patients; eGFR level correlates with severity of chronic kidney disease

Table 9. Laboratory Tests Used in the Management of Patients with Diabetes

Laboratory Test	Significant Concentration
Self-monitoring blood glucose (mg/d)	
Preprandial target	90-130
Peak postprandial target*	<180
Hemoglobin A_{1c} (%)	
Adults	<7 (<6†)
0-6 y	7.5-8.5
6-12 y	<8
13-19 y	<8 (<7†)
Lipids (mg/dL)	
LDL	<100
Triglycerides	<150
HDL	
Males	>40
Females	>50
Microalbumin (μg/mg creatinine)	
Normal	<30
Early nephropathy	30-299
Clinical nephropathy	≥300
eGFR (mL/min/1.73 m^2)	
Stage 1	≥90
Stage 2	60-89
Stage 3	30-59
Stage 4	15-29
Stage 5	<15

*1-2 hours after beginning a meal.

†Preferred target if no hypoglycemia.

From *Diabetes Care*. 2006:29(Suppl 1):S4-S42.

Hypoglycemic Disorders

The complexity of the glucose regulatory mechanisms provides for a heterogeneity of causes for clinical hypoglycemic syndromes. The diagnosis and differential diagnosis of these disorders are often difficult. Although there is some overlap among the types of hypoglycemia in children and adults, there are a variety of disorders unique to children (Table 10). These include the congenital glycogen storage diseases and deficiencies of gluconeogenic or fatty acid oxidation enzymes as well as congenital disorders involving the pancreas (infantile hypoglycemic hyperinsulinemia, IHH), pituitary, or adrenal glands. IHH has been associated with mutation of the sulfonylurea receptor on the pancreatic beta cell, altering control of insulin release. The disorder was previously referred to as nesidioblastosis.

Test Application and Interpretation

Any infant or child with plasma glucose under 50 mg/dL should be observed carefully, and a value below 40 mg/dL is considered hypoglycemia. Transient hypoglycemia in the newborn infant and, particularly, the premature neonate, is relatively common and usually resolves within 72 hours. The small-for-gestation-age infant and those with a predisposition to transient neonatal hyperinsulinemia (infant of diabetic mother, Beckwith-Wiedemann infant, infant with erythroblastosis fetalis) are at higher risk. Hypoglycemia persisting beyond 72 hours suggests a pathologic condition such as persistent hyperinsulinemia, which may be due to islet beta cell hyperplasia or IHH. Inborn errors of carbohydrate, amino acid, or organic acid metabolism usually are manifest in the newborn period. Pituitary or adrenal insufficiency usually is manifest at birth or during infancy. During childhood, the most common cause of hypoglycemia is ketotic hypoglycemia, a self-limited inability to maintain normoglycemia during prolonged fasting. Islet beta-cell hyperplasia or adenoma is uncommon and usually develops in later childhood.

In adults, the differential diagnosis is extensive and includes postprandial and fasting hypoglycemias. Postprandial hypoglycemia is common and has been classified into 3 types: patients with normal oral glucose tolerance tests (functional hypoglycemia), those with post surgical modifications of the upper GI tract (alimentary hypoglycemia), and those with early elevations (1 to 2 hours) of glucose levels during glucose tolerance testing (early diabetes hypoglycemia). The causes of fasting hypoglycemia (8 hours or more after feeding) include ethanol ingestion, factitious or accidental intake of oral hypoglycemic agents, severe liver disease, adrenal insufficiency, inanition, certain malignancies (leukemias, hepatoma, and mesenchymal tumors), and, rarely, insulin receptor stimulating autoantibodies. The prevalence of insulinoma approximates 1 patient per 250,000 patient-years. The median age at diagnosis is 50 years except for patients with multiple endocrine neoplasia (MEN) type I. Symptoms may present within weeks or decades before diagnosis.

Diagnostic Approach

In many adult cases, the diagnosis can be confirmed by demonstrating consistently inappropriate insulin concentrations in the presence of low blood sugar values. Insulin-like growth factor binding protein I (IGFBP-1) is insulin dependent, and elevated levels often are associated with hyperinsulinism. As in children, in some instances, diagnosis may require prolonged fasting. In patients with insulinoma, insulin concentrations are ≥6 μIU/mL and C-peptide is >0.8 ng/mL when the blood sugar falls to 50 mg/dL or less; normal subjects have lower values. In adult patients with insulinoma, Whipple's triad (low plasma glucose, symptoms of hypoglycemia, and amelioration of symptoms by glucose administration) is demonstrated in 95% to 98% of cases within 72 hours of initiating a fast, and blood glucose values are ≤46 mg/dL at the time of development of hypoglycemic symptoms. Patients with insulinoma usually have undetectable insulin antibody and an insulin/proinsulin plasma concentration ratio of 1:1, whereas in normal subjects, ratios approximate 6:1.

Table 10. Assessment of Fasting Hypoglycemia

Disorder	Blood Measurements								Other
	Insulin	C-Peptide	GH	Cortisol	FFA	Lactate	IGFBP-1	Urine Ketones	
Glycogen storage diseases	↓	↓	N	N	↑	↑	N	↑	↓ glucose response to glucagon; abnormal liver glycogen
Gluconeogenic enzyme deficiencies	↓	↓	N	N	↑	↑	N	↑	↓ glucose response to glucagon; abnormal liver enzyme
Galactosemia	↓	↓	N	N	↑	↑	N	↑	↑ blood/urine galactose; cataracts; failure to thrive
Hereditary fructose intolerance	↓	↓	N	N	↑	↑	N	↑	Specific fructose intolerance
Defective fatty acid oxidation	↓	↓	N	N	↑	↑	N	↓	↓ blood carnitine
Anti-insulin hormone deficiency									
GH	↓	↓	↓	N	↑	↑	N	↑	
Cortisol	↓	↓	N	↓	↑	↑	N	↑	
Infantile hypoglycemic hyperinsulinism*	↑	↑	N	N	↓	↓	↑	↓	↑ blood ammonia; leucine sensitivity
Islet-cell tumor or hyperplasia	↑	↑	N	N	↓	↓	↑	↓	
Activating glucokinase mutation	↑	↑	N	N	↓	↓	↑	↓	
Ketotic hypoglycemia	↓	↓	N	N	↑	↑	N	↑	↓ blood alanine
Organic acidurias	↓	↓	N	N	↑	↑	N	↑	Impaired gluconeogenesis
Liver disease	↓	↓	N	N	↑	↑	↑	↑	Severe metabolic acidosis; abnormal liver function tests

GH, growth hormone; FFA, free fatty acids; N, normal.
*Mutation of the glutamate dehydrogenase gene is associated with hyperammonemia and increased sensitivity to leucine-induced hypoglycemia.

Test Application and Interpretation

Various perturbation tests have been proposed for the diagnosis of insulinoma including the tolbutamide tolerance test, the glucagon stimulation test, and the C-peptide suppression test. However, all are associated with frequent false-positive or false-negative results. The most reliable confirmatory test is the classic 72-hour fast which produces hypoglycemia in essentially all patients with insulinoma; 75% develop hypoglycemia within 24 hours (see Dynamic Test section). Patients with nesidioblastosis may have postprandial hypoglycemia associated with abnormal plasma insulin and C-peptide levels.

The C-peptide suppression test is influenced by age and body mass index (BMI); suppressibility at 60 minutes decreases with increasing age and increasing BMI. Suppressibility in normal subjects aged 20 to 50 with BMI of 20 to 34 kg/M^2 ranges from 59% to 67%; values in subjects aged 51 to 80 range from 51% to 61%. These test protocols are summarized in the Dynamic Test section.

The insulin autoimmune syndrome (IAS) is an unusual cause of hypoglycemia associated with the spontaneous development of insulin autoantibodies in patients never exposed to exogenous insulin. These individuals often manifest other autoimmune diseases, including rheumatoid arthritis, and some have received penicillamine. The autoantibodies in these patients often interfere with insulin measurement by immunoassay, producing artifactually high values in the standard competitive RIA methods. Measurement of autoantibody levels can clarify the situation. The pathogenesis of hypoglycemia in IAS patients is thought to involve binding of large amounts of insulin and dissociation from antibody during periods of fasting.

The initial approach to the diagnosis of hypoglycemia in infants or children includes measurement of blood glucose, insulin, C-peptide, cortisol, growth hormone, IGFBP-1, liver function studies, plasma free fatty acids, and urine ketones. These studies should be obtained at the time of, and in the presence of, hypoglycemia. In the presence of a whole blood glucose level <40 mg/dL, the normal glucose/insulin ratio (mg/dL/μU/mL) exceeds 4/1, cortisol exceeds 10 mg/dL, and the growth hormone concentration exceeds 10 ng/mL. A low ratio suggests insulin excess; low levels of cortisol or growth hormone suggest deficiency. In many instances, the hypoglycemia is intermittent and manifest only during prolonged fasting. This is especially true in the rare patients with early, mild, inappropriate insulin secretion due to an islet cell adenoma. In such instances, prolonged fasting is helpful to confirm the diagnosis of hypoglycemia. IGFBP-1 is insulin dependent, and elevated levels often are associated with hyperinsulinism. In patients with insulinoma, insulin concentrations are ≥6 μIU/mL and C-peptide ≥0.6 ng/mL when the blood sugar falls to ≤40 mg/dL; normal subjects have lower values. Patients with insulinoma usually have undetectable insulin antibody and an insulin/proinsulin plasma concentration ratio of 1:1, whereas in normal subjects, ratios approximate 6:1.

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DISORDERS OF GONADAL FUNCTION

Background Physiology

Development of the Hypothalamic–Pituitary Axis and Isolated Gonadotropin Releasing Hormone (GnRH) Deficiency

As briefly discussed in the "Disorders of Anterior Pituitary Function" section, mutations in several transcription factors may impact development of the pituitary, including function of the gonadotropin-producing cells (Figure 1). Mutations that exclusively impact the reproductive system include the X-linked *KAL1* gene that encodes the protein anosmin-1. Anosmin-1 is a cell adhesion molecule that is responsible for the in utero migration of the GnRH and olfactory neurons from the olfactory placode to their final destination in the hypothalamus. *KAL1* mutations are associated with Kallmann syndrome, as are mutations in the fibroblast growth factor receptor 1 (*FGFR1* or *KAL2*) gene. *FGFR1* mutations appear in a subset of patients with Kallmann syndrome who lack *KAL1* mutations. FGFR1 appears to modulate the action of anosmin. These subjects may present with isolated anosmia, GnRH deficiency and anosmia, or dental agenesis and cleft palate. Some may be completely asymptomatic despite carrying a mutation. A third cause of isolated hypothalamic hypogonadism was shown in subjects with mutations in the GnRH receptor gene leading to diminished or absent pituitary gonadotropin secretion. Two other transcription factors, SF1 (renamed NR5A1) and DAX1 (renamed NROB1) also impact GnRH and gonadal and adrenal steroidogenesis. *DAX1* mutations in rodents appear less critical for hypothalamic–pituitary–gonadal axis function in infancy but are crucial in triggering GnRH and gonadotropin secretion during puberty. The phenotypes associated with these mutations depend on the sex of the proband and may be associated with adrenal insufficiency.

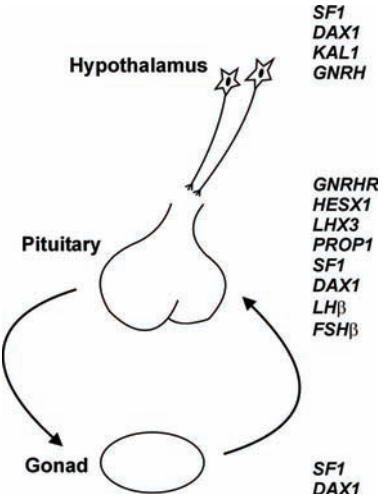

Figure 1. Transcription factors of the hypothalamic–pituitary–gonadal axis.

Test Application and Interpretation

Regulation of Pubertal Onset

The triggering of puberty is known to involve a complex interplay of stimulating and inhibiting inputs at the level of the hypothalamus. The molecular events mediating puberty onset are beginning to be unraveled. The G-coupled orphan receptor GPR54 and its ligand kisspeptin (metastin) encoded by the *KiSS-1* gene have been shown to increase dramatically in several species during puberty. Administration of kisspeptin in primates or rodents triggers puberty while mutation in the *GPR54* gene in humans leads to sexual immaturity. The upstream and downstream events remain unclear, but it is possible that kisspeptin and its receptor act to integrate the hypothalamic input, including feedback from nutritional cues conveyed by leptin, the gonadal steroids, and other environmental and metabolic factors, to trigger GnRH and gonadotropin secretion (Figure 2).

Regulation of the Mature Hypothalamic–Pituitary–Gonadal Axis

The hypothalamic–pituitary–gonadal axis in males and females may be represented as a closed-loop feedback control system (Figures 3, 4). The hypothalamus secretes pulses of GnRH to control pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), collectively known as gonadotropins. Pulsatility of GnRH secretion is critical for normal gonadal function, as constant administration of GnRH or GnRH analogues leads to eventual suppression of gonadotropin secretion.

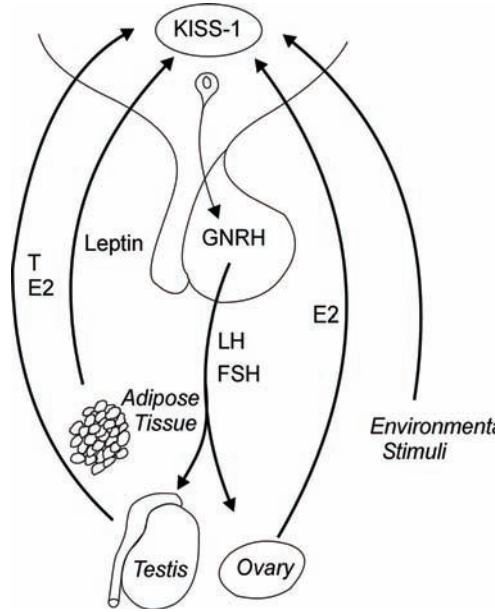

Figure 2. Genetic and hormonal factors involved in the onset of puberty.

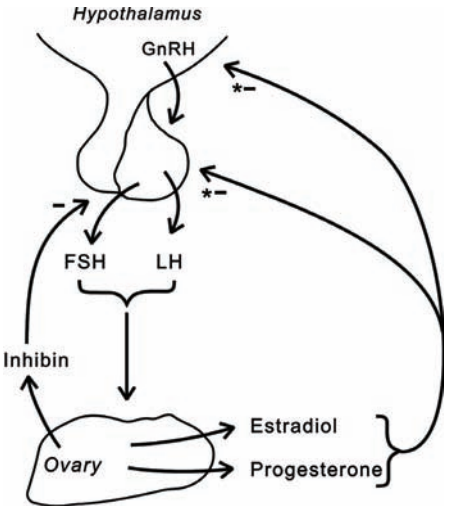

Figure 3. The female hypothalamic–pituitary–ovarian axis. GnRH, gonadotropin hormone releasing hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; –, negative feedback (inhibitory effect); *–, negative feedback (transient switch to positive feedback prior to ovulation).

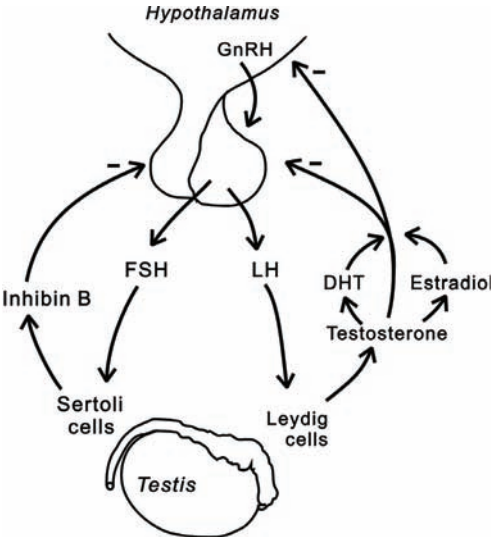

Figure 4. The male hypothalamic–pituitary–ovarian axis. GnRH, gonadotropin hormone releasing hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; DHT, dihydrotestosterone; –, negative feedback (inhibitory effect).

In females (Figure 3), LH stimulates androgen production by the thecal cells of ovarian follicles, while FSH induces the enzyme aromatase to convert theca cell-produced androgens to estrogens in ovarian granulosa cells. FSH also induces granulosa cell secretion of the polypeptide hormones inhibin A and B, which exert negative feedback on pituitary FSH release. Sex steroids (estrogens and progesterone, the dominant luteal phase hormone) secreted by the ovary exert negative feedback at the hypothalamic level. Estrogens also exert direct negative effects at the pituitary level. Midway through the normal menstrual cycle (Figure 5), the system transiently becomes a positive feedback system; thus, sex steroids stimulate, rather than inhibit, gonadotropin release. This switch from negative to positive feedback control leads to the mid-cycle surges of LH, FSH, and estradiol that precede ovulation.

In males (Figure 4), LH stimulates androgen production (primarily testosterone) by the Leydig cells of the testis, while FSH supports Sertoli cell functions critical for spermatogenesis. Sertoli cells produce inhibin B and inhibin A, which may be detectable in some, but not all, men with oligospermia. Both of these exert negative feedback on FSH release by the pituitary. Some circulating testosterone is converted in peripheral tissues to dihydrotestosterone and to small amounts of estradiol, and these sex steroids (particularly estradiol) exert negative feedback on the hypothalamus and pituitary. Both hormones have important male specific functions.

Knowledge of the negative feedback relationships of the hypothalamic–pituitary–gonadal axis is helpful in clinical diagnosis. For example, the patient with inadequate sex steroid production (hypogonadism) can be classified as either having elevated gonadotropins (hypergonadotropic hypogonadism, more likely

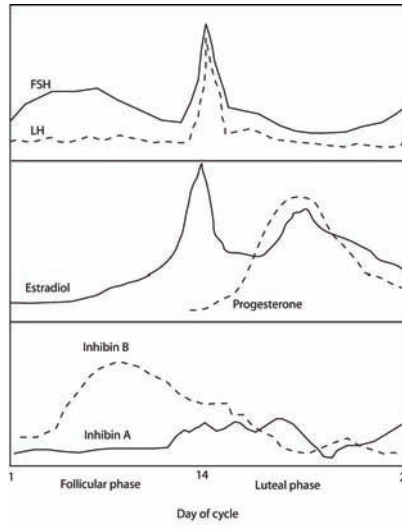

Figure 5. Hormonal changes during the menstrual cycle. Day 1, first day of menses. Note: duration of the menstrual cycle varies widely among healthy females (21-35 days).

a gonadal defect) or inappropriately low or normal gonadotropins (hypogonadotropic hypogonadism, suggestive of a hypothalamic–pituitary defect). An important exception to this general classification scheme arises in children between the ages of about 2 to 3 years until puberty; during these childhood years (Figure 6), the reproductive axis is quiescent, and gonadotropins will not rise, even when gonads fail or are completely absent (agonadal state).

Gonadal Determination and Differentiation

The primordial germ cells migrate from the epiblast to the base of the allantois and then to the urogenital ridge to form the bipotential gonad. An XY genotype of the embryo will lead to expression of the Y-linked sex reversal Y (*SRY*) gene within the urogenital ridge, which in turn triggers a genetic cascade leading to determination of gonadal differentiation. In humans, these events occur before the seventh week of pregnancy. In the absence of a Y chromosome or the *SRY* gene, the bipotential gonads become ovaries. Ovarian organogenesis was initially thought to occur by default in the absence of *SRY* expression, but this concept has recently been challenged. The sequential gene cascades leading to gonadal sex determination and subsequent differentiation into either a mature testis or ovary are complex. The bipotential gonad expresses a number of genes and gene products as detailed in Figure 7. Amongst these are *WT1* and *SF1*, thought to promote the expression of *SRY* in the male. Homeobox genes *SOX9* and *SOX8* are downstream of *SRY*. *SF1* regulates antimüllerian hormone (AMH,

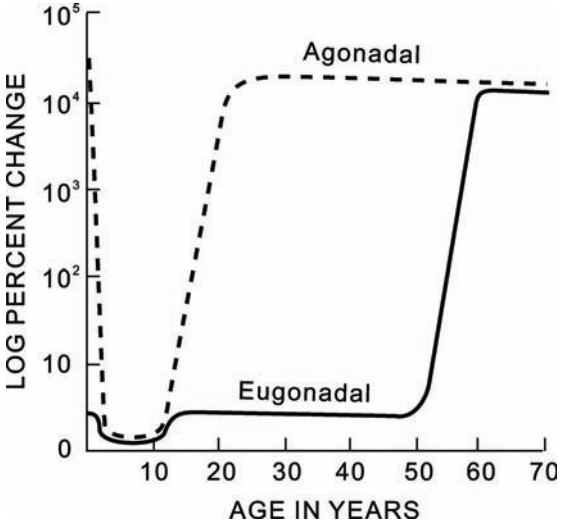

Figure 6: Schematic presentation of the percent change (log scale) in gonadotropins in agonadal (dotted line) and eugonadal (solid line) females from birth to 70 years. Between 2-3 years and puberty, the reproductive axis is quiescent.

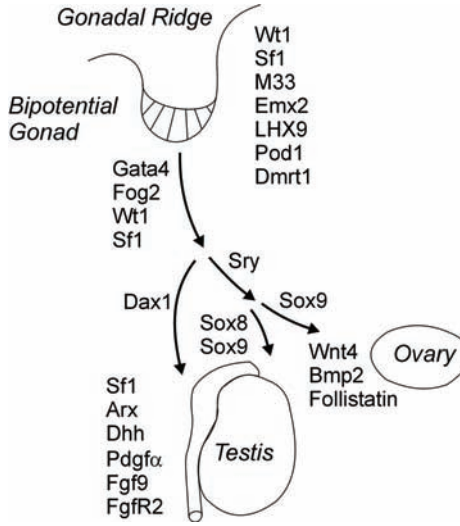

Figure 7: Sequential expression of factors governing sexual determination and differentiation. Abbreviations not shown in text: Wt1, Wilm's tumor suppressor; M33, transcriptional repressor member of polycomb family. Transcription factors: Emx2; Lhx9, lim homeobox; Pod1, podocyte; Gata4, Gata binding protein; Fog2, friend of Gata; Sox9, *SRY*-box 9; Arx, aristaless-related homeobox; Dhh, desert hedgehog; Pdgfα, platelet derived growth factor; Fgf9/FgfR2, fibroblast growth factor and fibroblast growth factor receptor; Wnt4, wingless/int; Bmp2, bone morphogenic protein; and follistatin. The function of these peptides is derived from the phenotype (such as sex reversal) they convey in genetically modified mice. Others have been implicated from their function in both rodents and humans.

or müllerian inhibiting substance [MIS]) activation in the Sertoli cells leading to regression of the müllerian ducts, while in Leydig cells *SF1* and *DAX1* act in concert to regulate steroidogenesis and testosterone production in utero. In the primordial testes, *DAX1* also contributes to the formation of testicular cords. Additional factors outlined in the same figure are involved in Leydig and Sertoli cell differentiation. Absence of *SF1* and AMH in the female leads to regression of the Wolffian ducts, while in the male the presence of testosterone sustains their development into the vas deferens, seminal vesicle, and prostate. Female differentiation requires the activity of *WNT4a* and repression of *SOX9*, *DAX1*, and other genes. Aberrant or lack of gene expression, along with mutations in genes encoding critical steroidogenic enzymes in utero, leads to a host of gonadal dysgenetic syndromes along with disorders of sexual differentiation. These are discussed further below.

Test Application and Interpretation

Measurement of Pituitary–Gonadal Axis Hormones

Luteinizing Hormone (LH, Lutropin)

LH consists of 2 subunits: an α-subunit (also found in FSH, TSH, and hCG) and a unique β-subunit. Competitive assays using polyclonal antibodies may demonstrate substantial cross-reaction with TSH and hCG due to presence of the common α-subunit. Such cross-reactivity is not seen with immunometric assays (eg, immunoradiometric [IRMA], immunochemiluminometric [ICMA], or immunofluorometric [IFMA] assays). Immunometric assays also tend to be more sensitive. The most sensitive (ultrasensitive, third generation, pediatric) LH assays better distinguish central precocious puberty (increased LH) from the prepubertal state.

The pulsatile release and the relatively short (60-minute) circulatory half-life of LH make a single LH measurement an unreliable estimate of the true serum LH concentration, which may easily be 50% to 150% of the single measured value. An apparently low level of LH derived from a single sample is therefore of limited clinical significance; it must be verified by repeat sampling and analysis.

Follicle Stimulating Hormone (FSH, Follitropin)

FSH, like LH, is a dimer comprised of a common α-subunit (same as for LH, TSH, and hCG) and a unique β-subunit. Unlike LH, FSH has a relatively long serum half-life (4-6 hours) and therefore shows less visible pulsatility. The major applications of FSH measurement are: 1) help differentiate pituitary from gonadal causes of infertility (anovulation or defects in spermatogenesis); 2) assess ovarian reserve (measurement of cycle day 3 FSH along with measurement of estradiol and inhibin B); and 3) detection and differential diagnosis of precocious puberty (along with measurement of ultrasensitive LH and sex steroid levels). While frequently used in the evaluation of menopausal symptoms, FSH is a surprisingly poor predictor of the exact timing of menopause.

Estrogens

In the non-pregnant woman, the endogenous estrogens of greatest significance are estradiol (E_2, 17β-estradiol) and the structurally similar, but less potent, estrone (E_1). Estriol (E_3) is the product of placental action on hormones secreted by the fetal adrenal gland and is therefore an important indicator of the health of the feto-placental unit. Being a thousand-fold less potent than estradiol, E_3 does not play a significant estrogenic role outside of pregnancy, however. More than 95% of circulating estrogens are bound to carrier proteins, including sex hormone-binding globulin (SHBG), cortisol-binding globulin (CBG), and albumin. SHBG is the most important. Its production in the liver is increased by estrogens and thyroid hormones and decreased by androgens and growth hormone. Testosterone competes with estrogens for SHBG binding sites. In patients with androgen excess or deficiency, hypo- or hyperthyroidism, pituitary dysfunction, liver disease, or gonadal dysfunction, the measurement of free estradiol may provide a more accurate assessment of bioavailable estrogen.

Test Application and Interpretation

Synthetic estrogens such as ethinyl estradiol (typically found in oral contraceptives) are not detected by most estradiol assays currently in use. Many "estradiol" assays still in use show significant cross-reactivity to estrone, which explains why they will detect a medication such as Premarin®' which is primarily comprised of horse estrogens (equilins) and estrone metabolites. Such assays can also be used to document the effect of oral 17β-estradiol, which is largely bioconverted to estrone in the intestinal mucosa. More recent estradiol assays show a much higher analytical specificity for estradiol. However, when maximal analytical sensitivity and specificity are needed (eg, during evaluation of gynecomastia or in prepubertal children) or when reported estradiol levels are not consistent with the clinical picture, the assay should be performed using LC/MS/MS technology. The LC/MS/MS assay is superior in its sensitivity and specificity relative to all immunoassays. Table 1 summarizes the 2 estradiol assays currently offered by Quest Diagnostics. E_1 and free E_2 can also be measured by LC/MS/MS.

Testosterone

There are 3 major categories of serum testosterone measurements: 1) total testosterone; 2) free testosterone, the portion not bound to sex-hormone binding globulin (SHBG) or albumin; and 3) free plus weakly-bound testosterone, ie, "bioavailable testosterone." The total testosterone measurement is crucial, since it is the basis for calculation of free and bioavailable testosterone. Thus, a false estimate of the total testosterone will translate to a false estimate of the free and bioavailable testosterone. When low values of testosterone are anticipated (eg, prepubertal males, all females) and when measuring levels in young infants in whom interfering substances are common, total testosterone is best measured by LC/MS/MS (or by RIA, preceded by extraction and chromatography if LC/MS/MS is unavailable). In both of these populations, the results derived from direct

Table 1. Characteristics of the Estradiol Assays Offered by Quest Diagnostics

	LC/MS/MS	Immunoassay
Test code	30289X	4021
Clinical use	Evaluate gynecomastia in prepubertal children	IVF protocols Monitor HRT (patch)
Cross-reactivity		
Conjugated equine estrogens (Premarin)	None	Minimal
Ethinyl estradiol	None	None?
Oral 17β-estradiol	None	Minimal
Transdermal 17β-estradiol	Yes	Yes
Aqueous interfering compounds	None	Some

LC/MS/MS, liquid chromatography tandem mass spectrometry; HRT, hormone replacement therapy; IVF, in vitro fertilization.

immunoassays (ie, those with no extraction or chromatography) often are unreliable. In adult males, total testosterone may be measured using non-extraction, direct immunoassay methods. There is a substantial bias in some of these assays compared with the LC/MS/MS method, and, while that might not matter in frankly hypogonadal males or in those with levels well within the normal range, it does matter in men with levels at the threshold of the diagnosis for male hypogonadism (300 ng/dL for young adults; 200 to 300 ng/dL in aging men). In such cases, a direct immunoassay might either under- or overestimate the number of males with mild clinical hypogonadism. Therefore, the Endocrine Society has recommended an extraction/chromatography RIA or LC/MS/MS measurement in these subjects (Ref. 27).

Measurement of bioavailable or weakly-bound testosterone can be performed using an ammonium sulfate precipitation method, or the levels can be calculated based on measurement of SHBG and albumin in addition to total testosterone. The former method shows very good correlation with the bioavailable testosterone levels obtained by calculation using SHBG and albumin.

Quantitation of free testosterone may likewise be obtained either by RIA after tracer equilibrium dialysis or by calculation based on SHBG, albumin, and total testosterone levels measured using either the RIA or LC/MS/MS method. While results from dialysis-based and calculated methods generally show reasonable equivalence, there are some situations (eg, high estrogen states such as pregnancy) in which the results from the 2 methods diverge.

Sex Hormone Binding Globulin

Levels of SHBG are known to drop with increasing androgen levels associated with advancing male puberty. Estrogens increase SHBG levels, but data from girls at various stages of puberty suggest that the increase in adrenal and gonadal androgens counterbalance any effect of increasing estradiol on SHBG levels in pubertal girls.

Inhibin

Elevated levels of inhibin A and, especially, inhibin B may be useful in detection of granulosa cell and mucinous carcinomas of the ovary, particularly in postmenopausal women whose inhibin levels are generally low. Measurement of inhibin B is also currently under investigation as an additional test (along with FSH and estradiol) of ovarian reserve for women entering an assisted reproduction program. For pediatrics, inhibin B may be of use in the diagnostic evaluation of cryptorchidism: peak inhibin values in male infants are generally seen at 3 months of age, making this an ideal time to identify those with anorchia (inhibin B levels more than 3 standard deviations below the mean) versus those with intra-abdominal testes.

Abnormalities of Sexual Determination and Differentiation

Our understanding of sexual differentiation and disorders thereof has expanded enormously during the past 3 decades. A useful approach is to divide these into 1) disorders related to defects in genetic factors that are critical in determining

the fate of the bipotential gonad before the seventh week of pregnancy, and 2) subsequent differentiation and steroidogenetic enzymatic defects that lead to ambiguous genitalia recognized during a newborn examination. The diagnostic capabilities for evaluating such infants have improved with our understanding of the complexity of the sexual differentiation process. Table 2 summarizes most of the disorders associated with sex reversal (discrepancy between chromosomal and phenotypic sex) and associated nonsexual developmental defects. Table 3 summarizes the steroidogenic enzyme defects and chromosomal abnormalities associated with ambiguous genitalia and laboratory tests that may be helpful in their evaluation.

Disorders of Sexual Maturation

Normal Puberty Staging

It is convenient to consider disorders of sexual maturation in 2 broad categories: those associated with early or precocious puberty and those associated with delayed or absent puberty. In any patient with abnormal sexual development, it is important to note whether the genitalia are normal and consistent with the genetic sex; ie, the phenotypic and genetic sex are identical. Often, determination of genetic sex requires diagnostic laboratory evaluation. The staging of normal puberty as outlined by Tanner is summarized in Table 4.

Timing of Physical Changes of Puberty

The normal range for puberty onset in girls has been somewhat controversial. Data from a large cross-sectional study of American girls suggest that the lower cutoff for puberty in white girls may be as young as 7 years (6 years for African American girls). Other data suggest that the ± 2 standard deviation (SD) range for breast development (thelarche) in white girls is approximately 8 to 13 years. The ± 2 SD for onset of menses (menarche) is approximately 10.5 to 15 years for white girls. On average, African American girls tend to have thelarche and menarche about 6 months earlier than do white girls.

For boys, the normal range for onset of testicular enlargement (testicular volume 3 mL or greater, or testicular length along the longest axis >1 inch) or genital enlargement has traditionally been 9 to 14 years, although some recent data suggest that a significant number of boys exhibit genital changes by 8 to 9 years of age.

Table 2. Genetic Disorders of Steroidogenesis and External Genitalia Differentiation

Gene	Mutant Phenotype
WT1	Frasier syndrome, Denys-Drash syndrome with Wilms tumor
SF1	Gonadal and adrenal dysgenesis
SOX9	Gonadal dysgenesis, congenital adrenal hypoplasia
SRY	Gonadal dysgenesis
AMH (MIS)	Persistent müllerian duct syndrome, type I
AMHR (MIS receptor)	Persistent müllerian duct syndrome, type II

Table 3. Assessment of Disorders Associated with Abnormal Sexual Differentiation

Disorder	Karyotype	External Genitalia	ACTH	Cortisol	Adrenal Androgen	Testosterone	DHT	Other
21-Hydroxylase deficiency*	XX	Amb	↑	↓ or N	↑	↑	↑	± salt wasting; see adrenal section
11-Hydroxylase deficiency*	XX	Amb	↑	↓ or N	↑	↑	↑	May be hypertensive
Aromatase deficiency	XX	Amb	N	N	N	N	N	↑ FHS/LH; maternal virilization
3β-HSD deficiency*	XY	Amb	↑	↓ or N	↓	↓	↓	± salt wasting; hypospadias
StAR deficiency	XY	F	↑	↓	↓	↓	↓	Salt wasting
17-Hydroxylase deficiency*	XY	F or Amb	↑	↓ or N	↓	↓	↓	Hypertension
17β-HSD deficiency	XY	F or Amb	N	N	N	↓	↓	↑ estrone
LH/hCG resistance	XY	Amb or F	N	N	N	↓	↓	Increase in LH; FSH normal
Turner syndrome	XO, XO/XX, Xq, Xp, Xr, Xi	F	N	N	N	↓	↓	↑ FSH, LH; streak gonads
Dysgenetic gonads	XY, XO/XY, XX, or XXY	Amb	N	N	N	↓	↓	*SF1, SRY, FOXL2* mutations, Denys-Drash syndrome, WAGR syndrome, camptomelic dysplasia
Sex chromosome mosaicism	XO/XY	Amb	N	N	N	↓	↓	Gonadal dysgenesis
True hemaphrodite	XX/XY, XX, or XY	Amb	N	N	N	↓	↓	Ovotestis, testis, ovary
Testicular feminization, partial	XY	Amb	N	N	N	N or ↑	N or ↑	Androgen receptor mutation; ↑ LH
5α-Reductase deficiency	XY	Amb	N	N	N	N or ↑	↓	↑ LH
Testicular regression syndrome	XY	M, Amb, or F	N	N	N	↓	↓	Absent müllerian duct derivatives and absent müllerian inhibiting hormone

ACTH, adrenocorticotropic hormone, DHT, dihydrotestosterone; Amb, ambiguous; N, normal; FSH, follicle stimulating hormone; LH, luteinizing hormone; HSD, hydroxysteroid dehydrogenase; F, female; M, male.

*Adrenal steroid precursor elevations are diagnostic.

Test Application & Interpretation

Table 4. Tanner Staging

Tanner Stage	Description
Breast	
B1	No visible breast enlargement
B2	Initial elevation of breast and papilla as a small mound
B3	Further enlargement of breast and areola, with a continuous rounded contour
B4	Areola and papilla form a secondary mound above primary breast mound
B5	Mature breast; rounded contour, with no areolar secondary mound present
Pubic Hair, Females	
PH1	No visible pubic hair
PH2	Sparse long, slightly pigmented, downy hair with minimum curl, chiefly on the labia
PH3	Darker, coarser, more curly pubic hair joining sparsely in the midline over the mons
PH4	Adult-type hair, but in a smaller area than in adults; no spread to medial thighs
PH5	Adult-type hair in the classic inverse triangle distribution; may spread to medial thighs
Genitalia, Males	
G1	Penis and testes the same size as during childhood. No scrotal thinning or reddening
G2	Enlargement of testes; thinning and reddening of scrotum
G3	Increase in penile length; further growth of testes and scrotum
G4	Further increase in both penile length and width. Further darkening of scrotal skin
G5	Adult male genitalia (adult size and configuration)
Pubic Hair, Males	
PH1	No pubic hair
PH2	Sparse long, slightly pigmented, downy hair with minimum curl, chiefly near the base of the penis
PH3	Darker, coarser, more curly pubic hair joining sparsely in the midline over the symphysis pubis
PH4	Adult-type hair, but in a smaller area than in adults; no spread to medial thighs
PH5	Adult-type hair in the classic inverse triangle distribution; may spread to medial thighs

Normal Variants of Puberty

Premature thelarche (early, unilateral or bilateral breast development in girls, without other physical or hormonal signs of puberty) is a common variant of normal and typically appears around 2 years of age, although some individuals may present with breast development from birth. For definitive diagnosis of premature thelarche, the following criteria should be met: 1) no acceleration of linear growth beyond normal for chronological age; 2) no appearance of pubic hair (pubarche); and 3) no significant acne. Skeletal age, as determined by a

bone X-ray of the left hand and wrist, should not be advanced beyond 2 SD relative to the chronological age. Extensive laboratory testing is generally not necessary if the presentation is relatively classic, but periodic clinical follow-up is recommended.

Premature adrenarche is the early appearance of pubic (occasionally axillary) hair, apocrine body odor, and mild acne. There should be: 1) no significant growth acceleration; 2) no other signs of androgen effect such as penile or clitoral enlargement; and 3) no breast development (thelarche) in girls or testicular enlargement in boys. As with premature thelarche, the skeletal age should not be significantly advanced (greater than +2 SD). There appears to be no significant sequelae in boys, but premature adrenarche in some girls may be an early harbinger of insulin resistance and the polycystic ovarian syndrome. If premature adrenarche is particularly rapid or "explosive" in onset, measurement of adrenal and gonadal steroids (eg, 17-hydroxyprogesterone, testosterone) should be considered to rule out entities such as congenital adrenal hyperplasia or adrenal/gonadal tumors. DHEA-sulfate levels are typically in the mid-pubertal range in premature adrenarche; much higher levels of DHEA-sulfate suggest a need for further testing, including imaging of the adrenals.

Precocious Puberty

There are a large number of causes of precocious sexual development (Table 5). As a general rule, precocious puberty in girls is more often idiopathic, while organic causes (eg, central nervous system lesions and tumors) are more likely in boys with true central precocious puberty.

Ultrasound examination of the pelvis in such girls provides information about ovarian and uterine size; uterine volumes of >1.8 mL and/or ovarian volumes >1.2 mL are consistent with onset of true puberty. Measurements of serum gonadotropin and estradiol levels and gonadotropin response to gonadotropin releasing hormone (GnRH) usually allow differential diagnosis of premature thelarche, central precocious puberty, and gonadotropin-independent precocity. LH and FSH usually are detectable and in the pubertal range in gonadotropin-dependent and low or undetectable in gonadotropin-independent precocious puberty.

In boys, measurement of serum gonadotropin and testosterone levels also is necessary, and if basal LH levels are low, GnRH stimulation will help differentiate gonadotropin-dependent and -independent precocity. The clinical suspicion of intracranial pathology in either sex requires cranial imaging. Treatment of central precocious puberty with long-acting GnRH analogues requires GnRH testing to assess treatment response.

Delayed or Absent Puberty

Delayed puberty is generally defined as the absence of pubertal sexual characteristics by age 13 in girls and age 14 in boys; a small percent (2.5%) of the normal population mature in later years. The major causes of delayed puberty are listed in Table 6. Each of these major causes may in turn be subdivided into multiple etiologies. For example, anorexia nervosa, starvation, athletic or exercise amenorrhea, and the rare zinc deficiency can all be related to the broad

Table 5. Causes of Premature Sexual Development

Gonadotropin-dependent
True precocious puberty
Familial precocious puberty
Cerebral neoplasms (hamartoma, germ cell tumor, glioma, colloid cyst)
Head injury
Cranial irradiation
Meningitis or encephalitis
Congenital hydrocephalus
Kabuki makeup syndrome
Hypothyroidism
Teratomas (pineal, extra CNS)
Hepatoblastoma
Pituitary tumor
Gonadotropin-independent
McCune-Albright syndrome
Familial male sexual precocity
Ovarian neoplasms (granulosa cell, cyst, adrenal rest)
Leydig cell neoplasms
Gonadoblastoma, sex cord tumors
Adrenal neoplasms
Virilizing congenital adrenal hyperplasia
Activating mutations of the LH receptor
Other
Premature thelarche
Premature adrenarche
Premature menarche
Exogenous steroids

area of nutritional disorders. Careful history and physical examination will often permit identification of the specific cause amongst these major categories. Nutritional disorders, hypothalamic and pituitary disorders, and familial or constitutional delay are associated with low blood LH and FSH concentrations and low levels of gonadal steroids indistinguishable from those seen in normal prepubertal children. It is often very difficult to differentiate constitutional delay in growth and sexual development (CDGSD) from hypothalamic/pituitary disorders unless an obvious intracranial mass is present or hyposmia, anosmia, or facial defects suggesting median facial cleft syndromes are evident. The difficulty in separating CDGSD from a hypothalamic/pituitary hypofunction that will persist is greatest in the 13-14 to 17-18 age range. Beyond this age period, as bone

Table 6. Causes of Delayed Sexual Development
Constitutional delay in growth and sexual development
Nutritional disorders
Hypothalamic disorders (GnRH deficiency)
Pituitary disorders (hypopituitarism or hyperprolactinemia)
Gonadal disorders (primary gonadal disorders)
Hyperandrogen disorders (females only)
Anatomic abnormalities

age matures and puberty still fails to occur, CDGSD becomes less likely. In primary gonadal disorders, once the hypothalamic changes of puberty occur (typically after age 10 years), blood LH and FSH concentrations rise to highly elevated castrate levels, making diagnosis fairly easy.

Disorders of Testicular Function

Developmental Disorders

Disorders of testicular function are summarized in Table 7. Clinically, abnormalities range from mild abnormalities in androgen secretion or action to 46,XY genetic males that appear as virtually normal females. When complete, enzymatic defects in testosterone biosynthesis and defects in androgen action (eg, androgen resistance, inactivating LH receptor mutations) give rise to individuals with female external genitalia (male pseudohermaphroditism). Depending on the severity of the defect in biosynthesis or androgen action, the genitalia may range from nearly normal female to normal male. If androgen deficiency occurs after embryogenesis is completed, but before the onset of puberty, an eunuchoid individual is expected. In such individuals, long bone growth is prolonged because of delayed epiphyseal fusion. The result is a longer arm span and longer legs than in normal individuals. Height is expected to be equal to arm span in most post-pubertal males. In eunuchoid males, arm span is greater than height, usually by several inches. If androgen deficiency is acquired after normal embryogenesis and normal puberty, a hypogonadal male with decreases in reversible androgen effects, eg, decreased beard, decreased or absent axillary and pubic hair, and decreased muscle strength, is expected. Thus, it is generally possible to date the time of onset and to limit the diagnostic possibilities based on the clinical findings. It is always important to consider the possibility that genetic abnormalities may be the basis for the hypogonadism.

Disorders of the Germinal Cells

Male factor infertility affects 5% to 10% of the male population and presents as a disorder of spermatogenesis that is not associated with a hormonal abnormality, indicating preservation of the steroidogenic compartment. In 10% to 15% of the cases, this could be due to a genetic abnormality including deletions on the long arm of the Y chromosome. Most involve deletion of several

Table 7. Differential Diagnosis of Testicular Function Disorders

Disorder	Diagnostic Features
Abnormalities of hypothalamic-pituitary function	Normal or ↓ FSH, LH
Panhypopituitarism	↓ testosterone
GnRH deficiency (Kallmann's syndrome)	Genetics – 46,XY
Isolated LH or FSH deficiency	Genitalia – male
Hyperprolactinemia syndromes	
Prader-Willi syndrome	
Vasquez-Hurst-Sotos syndrome	
Laurence-Moon-Biedl syndrome	
Cerebellar ataxia	
Neoplasms (eg, craniopharyngioma, meningioma, astrocytoma)	
Granulomatous disorders (eg, histiocytosis)	
Primary gonadal abnormalities involving androgen production	↑ LH and/or FSH ↓ testosterone
XX males (sex reversal syndrome)	Genetics – variable
True hermaphroditism	Genitalia – male
Streak gonads – XY karyotype	
Testicular agenesis (vanishing testes syndrome)	
Noonan's syndrome	
Klinefelter's syndrome	
Acquired testicular disorders: orchitis, surgical castration, etc.	
Chemotherapy	
Leydig cell hypoplasia	
Enzymatic defects in androgen synthesis	↑ LH, FSH
20α-Hydroxylase deficiency (lipoid adrenal hypoplasia)	↓ testosterone
17,20-Desmolase deficiency	Genetics – 46,XY
3β-Hydroxysteroid dehydrogenase deficiency	Genitalia, ambiguous
17-Hydroxylase deficiency	
17-Ketosteroid reductase deficiency	
5α-Reductase deficiency	
Defects in androgen action	Normal LH, FSH
Complete androgen insensitivity (testicular feminization)	Normal or ↑ testosterone
Incomplete androgen sensitivity	Genitalia, depending on severity in defects, ranges from nearly normal female to normal male
Miscellaneous causes	
Persistent müllerian duct syndrome	
Cystic fibrosis	

copies of the *DAZ* (deleted in azoospermia) gene. *CFTR* (cystic fibrosis) mutations lead to congenital absence of the vas deferens and subsequent infertility. Androgen receptor mutations have been documented in azoospermic individuals who appear well androgenized and have normal testes size. The only hint to the diagnosis is slightly elevated LH and testosterone levels. The most frequent sex chromosome aneuploidy leading to azoospermia is Klinefelter syndrome (XXY) with a prevalence of 0.1% to 0.2% in the general population and 3.1% in the infertile population. The disorder primarily leads to early failure of spermatogenesis followed by a subsequent failure of steroidogenesis presenting as primary hypogonadism. The symptoms of hypogonadism might be subtle and delayed as testosterone may be maintained in the lower normal range at the expense of an elevated LH. Depending on the degree of hypogonadism, it might come to attention as delayed puberty in a subject with a eunuchoidal body habitus and small testes or as male factor infertility associated with azoospermia. Mutations in the *Insl3* (insulin-like factor 3) gene have been reported in 5% to 10% percent of all subjects with cryptorchidism. With the advent of intracytoplasmic sperm injection (ICSI), it is now possible for many couples with an affected male partner to father a child. Determining presence of an abnormal karyotype or the *Insl3* mutation prior to *ICSI* may be of value for genetic counseling purposes, as the newborn may also be affected and infertile. This is especially relevant in men with mild cystic fibrosis mutations, leading to congenital absence of the vas deferens as the sole manifestation of the disease, and in subjects with Klinefelter syndrome.

Testicular Aging

The steroidogenic capacity of the testes decreases with age, and there is a gradual decline in testosterone levels below the lower cutoff of the young adult range. This decrease is usually not associated with an increase in LH and FSH, indicating that reproductive aging occurs both at the testicular and pituitary levels. This testosterone decrease becomes more pronounced each decade, and its extent depends on the baseline from which the testosterone level declines in each individual. This decrease may be associated with increased frailty, decreased muscle mass, osteoporosis, and an increase in cardiovascular risk in the aging population collectively labeled as the andropause. It is not clear whether the decrease in testosterone in older men, while observationally correct, is actually physiologic. Some preliminary, small, testosterone replacement trials show some benefit in older men: increased muscle mass, and possibly strength, along with improvement in bone mineral density. A large multicenter efficacy trial of testosterone in older men mandated by the Institute of Medicine and funded by the National Institutes of Health is under way. Primary endpoints include muscle strength, cardiovascular biomarker levels, bone mineral density, and bone turnover marker levels. Secondary end points are related to safety of replacement therapy such as increased risk of benign prostatic hyperplasia and possibly prostate cancer. In the interim, and until the results of this and other trials become available, the ideal reference range of testosterone for the aging men remains a subject of debate. The Endocrine Society consensus guidelines on the management of hypogonadism, and similar guidelines from the American Association of Clinical Endocrinologists, indicate that men with persistent hypogonadal symptoms and low testosterone levels (ie, below the lower cutoff for

young men) on more than one occasion may benefit from testosterone replacement after adequate counseling about the risks and benefits of such therapy. In these consensus statements, there was disagreement on the level of testosterone below which testosterone therapy should be instituted, but there is agreement that levels ≤200 and 320 ng/dL warrant therapeutic intervention when associated with signs and symptoms of andropause.

Disorders of Ovarian Function

Ovarian agenesis or hypofunction is not associated with abnormal sexual differentiation, but may be associated with morphologic anomalies and slow growth during the prepubertal period. Ovarian dysfunction is associated with delayed puberty and with oligo or amenorrhea. Tables 8 and 9 summarize the

Table 8. Differential Diagnosis of Primary Amenorrhea

Disorder	Diagnostic Features
Abnormalities of hypothalamic–pituitary function	↓ or normal FSH, LH
Panhypopituitarism	↓ Estrogen
GnRH deficiency (Kallmann's syndrome)	Genetics – 46,XX
Isolated LH or FSH deficiency	Genitalia – female
Hyperprolactinemia syndromes	
Neoplasms (eg, craniopharyngioma)	
Granulomatous diseases (histiocytosis X)	
Exercise/nutritional related	
Primary gonadal abnormalities	↑ FSH, LH
Turner's syndrome	↓ Estrogen
Gonadal dysgenesis	Genetics abnormal (see Table 3)
Defects in androgen/estrogen production or action	Normal FSH, LH
Androgen resistance (testicular feminization)	↓ to ↑ testosterone, depending on defect
Enzymatic defects in androgen synthesis	
20α-Hydroxylase deficiency	Genetics – 46,XY
17,20-Desmolase deficiency	
3β-Hydroxysteroid dehydrogenase deficiency	
17-Hydroxylase deficiency	
17-Ketoreductase deficiency	
5α-Reductase deficiency	
Abnormalities leading to excess androgen production	↑ testosterone
CAH, 11-hydroxylase deficiency	Genetics – 46,XX
CAH, 21-hydroxylase deficiency	
Abnormalities of uterus and vagina	Normal FSH, LH
Vaginal agenesis with uterine abnormalities (Mayer-Rokitansky-Küster-Hauser syndrome)	Normal estrogen Genetics – 46,XX

Table 9. Differential Diagnosis of Secondary Amenorrhea

Disorder	Diagnostic Features
Pregnancy	↓ or normal FSH, LH
Gestational trophoblastic neoplasms	↑ hCG
Abnormalities of hypothalamic–pituitary function	↓ or ↓ normal FSH, LH
Pituitary adenoma	↓ or ↓ normal estrogen
Hypothalamic neoplasms	
Granulomatous diseases	
Miscellaneous diseases (eg, histiocytosis X)	
Hemosiderosis	
Hyperprolactinemia syndromes	
Systemic illness/nutritional/psychological	
Primary gonadal abnormalities	
Oophorectomy	↑ FSH, LH
Autoimmune oophoritis	
Radiation to ovaries	
Ovarian tumors associated with steroid hormone production	↓ or ↓ normal FSH, LH ↑ steroid hormones
Disorders of excess androgen production	
Polycystic ovarian syndrome	Normal FSH, LH
Ovarian neoplasms, androgen producing	↑ testosterone
Adrenal neoplasms producing androgens	
Idiopathic hirsutism	↑ DHEA-sulfate, androstenedione, testosterone
Cushing syndrome	

differential diagnosis of "primary" (never having had a menstrual period) and "secondary" (occurring following some time of regular menses) amenorrhea. The disorders causing primary and secondary amenorrhea overlap. The most common cause of secondary amenorrhea is normal pregnancy and the breast-feeding which follows. In subjects with primary amenorrhea, it is essential to correctly assign genetic sex, to note whether normal female secondary characteristics have occurred, and to note whether any systemic disease or androgen excess is present.

Premature Ovarian Failure (POF)

POF is defined as a premature demise in ovarian function in the context of an XX genotype. Most of the time, this leads to primary amenorrhea. On occasion, the onset may be delayed and present as secondary amenorrhea. POF may occur as an autoimmune disease in the context of multiple glandular insufficiency type I or type II. In type I, also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), the genetic defect has been traced to

mutations in the transcriptional activator autoimmune regulator gene (*AIRE*). Autoimmune ovarian failure may also be associated with type II polyglandular insufficiency, although no genetic abnormality has been identified for this syndrome. When not associated with other autoimmune disease, POF is also known as XX gonadal dysgenesis. Affected females have no other dysmorphic features, and their clinical presentation is limited to the consequences of early ovarian failure, streak ovaries by ultrasound, and elevated LH and FSH levels. There are candidate loci on the long arm of the X chromosome associated with this disease (*POF1* and *POF2*). The strongest evidence for the *POF1* locus is for the fragile X mental retardation 1 (*FMR1*) gene, followed by an X chromosome deletion that disrupts three candidate genes: heparan sulfate 6-O-sulfotransferase (*HS6ST*), *E2F*-related transcription factor, and glypican 3 (*GPC3*). There is also evidence implicating 2 other genes on the *POF2* locus that affect 3 other candidate genes: diaphanous homolog 2 (*DIAPH2*), dachshund homolog 2 (*DACH2*), and premature ovarian failure, 1B (*POF1B*). The most prominent autosomal gene implicated in this phenotype is the FSH receptor. A growing list of other genes is constantly added to this repertoire and has been recently reviewed by Fassnacht et al (Ref. 22).

Polycystic Ovarian Syndrome (PCOS)

PCOS is extremely prevalent amongst females of all ages and leads to important reproductive (infertility) and nonreproductive comorbidities. It is also associated with an increased risk of cardiovascular disease. The etiology is not clear and is probably multifactorial, but there is familial clustering of such cases. PCOS presents with menstrual irregularities ranging from secondary amenorrhea to oligomenorrhea. It is also associated with increased adrenal and ovarian androgen production, which leads to varying degrees of hirsutism including male pattern baldness, acne, and, occasionally, virilization. The ovaries are polycystic with associated failure of ovulation and absent corpus luteum formation. Progesterone secretion is decreased, leading to unopposed estrogen action with resultant endometrial hyperplasia and increased risk of endometrial cancer. PCOS frequently occurs in obese women. It is felt that the insulin resistance in such women may be at the center of the reproductive abnormalities, since increased insulin levels may increase androgen production in the ovarian theca cells.

A consensus conference convened in Rotterdam in 2003 established the minimal diagnostic criteria and work-up required for the diagnosis of PCOS. Patients need to fulfill 2 of 3 criteria: 1) presence of polycystic ovaries (with predefined morphologic features) on an abdominal or transvaginal ovarian ultrasound; 2) oligo- or anovulation; 3) clinical or biochemical evidence of hyperandrogenism. Exclusion of other causes of polycystic ovaries, while not stated as a criterion, is implicit. Recent guidelines by the Androgen Excess Society differ somewhat as they combine the first and second criteria into one, add hyperandrogenism (clinical or biochemical) as an essential component, and add exclusion of other conditions leading to hyperandrogenism as a third criterion. This definition excludes women who have oligo-amenorrhea and polycystic ovaries but no hyperandrogenism from the diagnosis.

Many other diseases may masquerade as PCOS. These include Cushing's disease, abnormalities in thyroid function, nonclassical 21-hydoxylase deficiency, hyperprolactinemia, and adrenal or ovarian virilizing tumors. The diagnostic workup should include laboratory tests guided by the clinical presentation. A minimum laboratory workup includes:

- Total and free testosterone, as free testosterone is more sensitive in detecting biochemical hyperandrogenism. The recommended method(s) for total testosterone is LC/MS/MS or a chromatography/ extraction RIA, since the analogue assays are highly inaccurate in this context. Free testosterone can then be determined by either the calculation or dialysis method.
- DHEA-S (sole androgen elevated in 10% of all cases)
- Tests aimed at ruling out other entities: prolactin, 17-OH progesterone, and TSH; 24-hour cortisol or estradiol, along with LH and FSH, are not necessary unless there is strong clinical suspicion of Cushing's disease or hypogonadotropic hypogonadism. The LH to FSH ratio usually is increased in PCOS but is highly variable and is not useful as a diagnostic criterion.
- Measures of insulin resistance such as a fasting glucose, OGTT, and a fasting lipid profile should be performed. Dietary modification, a decrease in body weight, or metformin therapy may lead decreased androgen production and resumption of ovulation and menses.

Others tests may be useful but are not universally endorsed. These include androstenedione, glucose/insulin ratio, or homeostatic model assessment of insulin resistance (HOMA-IR) for diagnosis of insulin resistance. It is important to make the diagnosis of PCOS, not only because of its impact on reproductive function, but because of the opportunity it provides for early intervention designed to prevent long-term cardiovascular and metabolic comorbidities.

Assessment of Functional Gonadal Tumors

Nearly all of the cell types in the testis and ovary can undergo neoplastic transformation. The majority of testicular neoplasms, however, are germ cell tumors that account for 90% to 95% of testicular cancer. These tumors originate early in life as pre-invasive precursors that transform into overt tumors during young adulthood. Such tumors may be as frequent as 1 in 10,000 young white males. Other testicular tumor types, and the usual hormonal changes, are summarized in Table 10.

Ovarian tumors with endocrine manifestations constitute <5% of all ovarian neoplasms and <10% of malignant ovarian cancer. However, as many as 50% may produce estrogens at a subclinical rate as evidenced by maturation in vaginal smears from postmenopausal women with ovarian cancer. Sex cord stromal tumors (those with granulosa cells, theca cells, Sertoli cells, or Leydig cells singly or in combination) are the neoplasms most frequently associated with the endocrine manifestations shown in Table 10.

Table 10. Assessment of Functional Gonadal Tumors

Disorder	LH	FSH	Testosterone	DHT	Other
Functional testicular tumors					
Germinoma	↓	↓	↑	↑	↑ hCG
Choriocarcinoma	↓	↓	↑	↑	↑ hCG
Leydig cell	↓	↓	↑	↑	↑ E_2
Sertoli cell	↓	↓	↓	↓	
Functional ovarian tumors					
Granulosa/theca cell	↓	↓	↑	N	↑ progesterone, inhibin
Androblastomas	↓	↓	↑	↑	
Dysgerminomas	↓	↓	↑	↑	↑ hCG
Choriocarinomas	↓	↓	↑	↑	↑ hCG
Gonadoblastomas	↓	↓	↑	↑	Abnormal karyotype
Struma ovarii	N	N	N	N	N or ↑ T4; ↓ TSH

DHT, dihydrotestosterone; E_2, estradiol; N, normal.

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DISORDERS OF THE GROWTH HORMONE-–INSULIN-LIKE GROWTH FACTOR AXIS

Background Physiology

Growth hormone (GH, somatotropin) is a single chain polypeptide hormone that is produced by the somatotrophs in the anterior pituitary gland. The major circulating form of GH (~75%) is 22 kd (191 amino acids) but a 20 kd form, resulting from alternative splicing of the primary mRNA transcript, is also present in the circulation. GH is secreted from the pituitary in a pulsatile pattern with a diurnal rhythm. Secretion is largely regulated by hypothalamic growth hormone releasing hormone (GHRH) and somatotropin releasing inhibiting factor (SRIF or somatostatin), which, respectively, stimulate and inhibit GH release (Figure 1). These neurotropic agents are, in turn, modulated by higher central nervous system (CNS) input and short loop feedback from GH and the insulin-like growth factors (IGF-I and IGF-II). CNS neuromediators include 1) dopamine and noradrenaline, which stimulate the release of both hypothalamic factors; 2) histamine and serotonin, which stimulate GHRH secretion; and 3) acetylcholine, which inhibits SRIF release. A variety of neuropeptides also have been shown to be capable of stimulating GH release. These include thyrotropin-

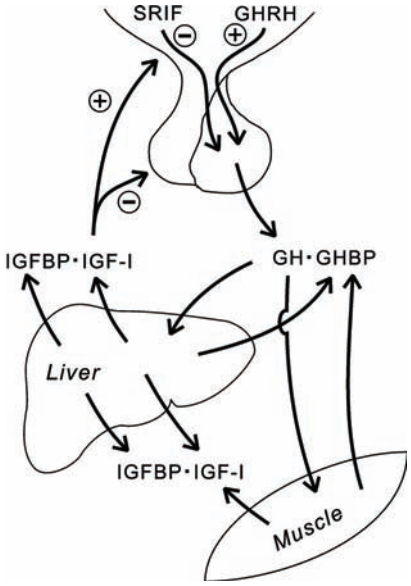

Figure 1. Features of the hypothalamic-pituitary-GH-IGF axis. SRIF, somatostatin; GHRH, growth hormone releasing hormone; IGFBP, IGF-binding proteins; IGF-1, insulin-like growth factor-1; GH, growth hormone; GHBP, growth hormone-binding protein.

releasing hormone (TRH), corticotropin releasing hormone (CRH), vasoactive intestinal peptide (VIP), gastrin, neurotensin, substance P, and several hypothalamic peptides (galanin and pituitary adenylate cyclase activating peptides, PACAP). A number of synthetic growth hormone releasing peptides (GHRP-1, GHRP-2, GHRP-6) also have been described. These peptides act via a distinct 7 transmembrane, G-protein coupled receptor termed the GH secretagogue receptor (GHSR). Recently it has been reported that the endogenous ligand for the GHSR is ghrelin, a 28 amino acid peptide produced primarily in gastric mucosal neuroendocrine cells. In normal subjects ghrelin levels increase during fasting and decline rapidly with feeding, suggesting that this hormone may play a critical role linking energy intake and GH secretion. Physiological factors known to stimulate GH release, presumably via one or more of the above mechanisms, include hypoglycemia, exercise, feeding, certain amino acids, and stage IV sleep. The diagnostic tests for assessment of GH secretory capacity (insulin-induced hypoglycemia, arginine infusion, alpha-adrenergic agonist stimulation by clonidine, L-dopa stimulation, vigorous exercise, sleep monitoring) acutely activate one or more of these pathways.

At physiologic concentrations, approximately half of the circulating GH is bound to the GH-binding protein (GHBP), a molecule identical to the extracellular domain of GH receptor. In humans, the GHBP appears to be produced by cleavage of membrane-bound GH receptor rather than by direct synthesis via alternative mRNA splicing, as occurs in rodents. In humans, GHBP levels correlate inversely with 24 hour GH production but are also responsive to estrogen and testosterone, particularly when administered orally. GHBP blood concentrations increase progressively during childhood and correlate with growth rate and adult height. The binding affinity of GHBP is such that about 50% of GH is bound at GH concentrations <10 ng/mL; GHBP saturation increases with increasing GH levels.

GH acts via plasma membrane GH receptors (GHR) in the liver and other tissues. The mature GHR has 620 amino acids and is composed of an extracellular hormone binding domain of 246 amino acids, a single transmembrane domain, and an intracellular domain of 350 amino acids. Binding of GH activates the cytoplasmic tyrosine kinase JAK2 which then phosphorylates the GHR, the transcription factors STAT 1, 3, and 5b, and other proteins involved in signal transduction. The major target genes regulated by growth hormone include those for insulin-like growth factor-I (IGF-I), IGF binding protein-3 (IGFBP-3), and the acid-labile subunit (ALS) that associates with the IGF-I:BP-3 complex.

The IGFs, IGF-I and IGF-II, are single chain polypeptide hormones that are structurally similar to each other (62% amino acid homology) and to insulin (50% homology). The IGFs are often referred to as somatomedins because they mediate many of the anabolic and mitogenic actions of GH (the somatomedin hypothesis). IGF-II is expressed at relatively high levels in the fetus and is believed to be a major regulator of fetal growth. The IGF-II gene is imprinted, with expression of the maternal allele being suppressed. IGF-II is also expressed postnatally in humans but not in mice or rats. Levels decrease rapidly after birth. IGF-I is expressed at low levels during fetal development, but the blood levels increase throughout gestation, reaching about 35% of adult levels at term. Postnatally, IGF-I is expressed in many tissues and, unlike IGF-II, its expression is

under relatively tight GH control. IGF-I secretion is also nutritionally regulated and age-dependent. In addition to its anabolic and mitogenic actions, IGF-I also inhibits apoptosis and exerts a negative feedback effect on GH release via actions at both the hypothalamic and pituitary levels (Figure 1).

The biological actions of the IGFs are mediated by the type I IGF receptor (IGFIR). This receptor is a transmembrane tyrosine kinase that is structurally similar to the insulin receptor. Binding of either IGF-I or IGF-II to the IGFIR results in tyrosine phosphorylation of intracellular target proteins, including members of the IRS family and Shc, and activation of intracellular anabolic and mitogenic pathways. IGF-II, but not IGF-I, also binds with high affinity to the type II IGF receptor; this receptor, which is identical to the mannose-6-phosphate receptor, is not anabolic and functions to clear IGF-II from the tissues. Interestingly, the type II IGF receptor is also imprinted, but in this case, expression of the paternally derived allele is suppressed. IGF-II is produced by certain tumors, particularly sarcomas, and can cause tumor-associated hypoglycemia.

The IGFs circulate in blood bound to several binding proteins (IGFBPs). Six distinct IGFBPs have been characterized, and 4 have been detected in human blood. They are glycoproteins whose core proteins range in size from 25 to 31 kd. Ninety-five percent to 99% of the circulating IGFs are bound to the binding proteins; only 1% to 5% circulates in unbound form. Seventy-five percent to 80% of the IGFs circulates in blood as a 150-kd complex comprised of IGF-I or IGF-II, IGFBP-3, and an 88-kd protein termed the acid labile subunit (ALS). IGFBP-3 and ALS are produced mainly by the liver. Synthesis of IGFBP-3 and ALS are stimulated by GH so that levels are increased in patients with acromegaly and decreased in those with growth hormone-deficiency. IGFBP-1, -2, and -4 also circulate in blood and bind most of the IGF not bound by IGFBP-3. All of the binding proteins inhibit IGF activity by competing with the IGFIR for the hormone, but IGF enhancing and IGF independent activities have also been reported for specific binding proteins and cell types.

The IGFs are synthesized by many cell types and tissues and act via both endocrine and autocrine/paracrine pathways. Circulating IGFs are derived mainly from hepatic synthesis. Mice in which the IGF-I gene was deleted selectively in the liver demonstrated marked reduction in circulating IGF-I concentrations and increased GH levels but normal growth, emphasizing the importance of autocrine/paracrine pathways in the growth response.

The major biological effect of GH is the stimulation of anabolic processes: linear growth during childhood and adolescence and maintenance of body composition and organ function in adults. Most of these effects are mediated through IGF-I. GH has little or no effect in the fetus because of a paucity of GH receptors. The growth effect becomes manifest during the early postnatal months and continues until epiphyseal closure. GH also has direct metabolic effects, including effects on insulin secretion and carbohydrate metabolism that are not mediated by the IGFs; large doses of GH paradoxically impair carbohydrate tolerance in spite of increased insulin secretion. GH deficiency is associated with increased fat deposition. In addition to its generalized anabolic activity, GH also

has tissue-specific anabolic effects. For example, GH (by stimulating local production of IGF-I) works synergistically with gonadotropins to stimulate steroid biosynthesis in the gonads.

Growth Hormone Deficiency in Children

GH deficiency can occur at any age. In children, the hallmark of GH deficiency is a subnormal growth velocity, resulting in short stature and delayed physical development. However, a large variety of non-GH factors impact growth, including genetic endowment, availability of calories and nutrients, systemic diseases, and other hormones, notably thyroxine, gonadal steroids, cortisol, and vitamin D. In practice, the vast majority of slow growing children are not GH deficient, and the differential diagnosis of growth failure is long. A thorough history, including a review of growth records, and physical examination are essential components of any work up of a short child. Routine laboratory studies including serum electrolytes, creatinine, calcium, liver function tests, blood count, ESR, urine analysis, and thyroid function tests should be obtained to rule out non-GH causes of poor growth. The measurement of IGF-I and IGFBP-3 are warranted if GH deficiency is a possibility. A bone age examination is often helpful for determining etiology and estimating growth potential. In short girls, a karyotype should be considered because gonadal dysgenesis may be causing the short stature.

GH deficiency can be congenital or acquired and can be isolated or involve multiple pituitary hormones. Congenital GH deficiency is an uncommon disorder having a prevalence of 1 in 4,000 to 1 in 10,000 births. Documented causes include hypothalamic and/or pituitary malformations, mutations affecting genes required for pituitary development (*PIT1*, *PROP1*, *HESX1*, etc.), hypothalamic GHRH deficiency, GHRH or GHRH receptor gene mutations, and GH gene mutations/deletions. In addition, the GH deficiency phenotype can be caused by primary GH resistance attributable to mutations/deletions in the genes for the GH receptor, members of the GH signal transduction pathway, IGF-I, ALS, or the IGFIR.

Acquired forms of GH deficiency are more common than congenital forms. These include GH deficiency resulting from CNS tumors (particularly craniopharyngiomas), inflammatory processes (sarcoidosis, histiocytosis), and radiation or trauma to the hypothalamus and/or pituitary. Autoimmune hypophysitis with antibodies directed at the pituitary somatotrophs has also been described in children and adults with apparent acquired GH deficiency and appears to be more common in subjects with isolated GH deficiency. Any of these etiologies may produce a complete or partial GH deficient phenotype so that diagnosis and differential diagnosis is difficult.

There is suggestive evidence in some short, slow growing children for a syndrome characterized by inadequate spontaneous GH secretion but normal responsiveness to pharmacological stimulation tests (hypothalamic neurosecretory dysfunction). To distinguish such patients, measurement of integrated GH secretion has been proposed (ie, frequent plasma GH measurements over a 12-hour (overnight) or 24-hour period). There is no consensus regarding the frequency of sampling and the parameters to be assessed

(area under the curve, sum of peak heights, mean concentration). Moreover, inter-individual reproducibility may be as high as 30%; thus, this approach has remained investigational. Urinary growth hormone measurement also remains investigational.

Growth Hormone Deficiency in Adults

It is estimated that 50,000 adults have GH deficiency and that each year 6,000 new cases are diagnosed in the United States. In adults, the signs and symptoms of GH deficiency can be subtle. They include increased adipose tissue (especially visceral fat); decreased lean body mass, bone mass, cardiac contractibility and exercise capacity; and alterations in mood and motivation. These features, along with osteopenia and increased risk of overall and cardiovascular mortality due to dyslipidemia and a proinflammatory state, led the FDA in 1996 to approve recombinant GH treatment for GH deficient adults. It is uncertain, however, if GH therapy leads to a decrease in cardiovascular mortality.

The term "somatopause" has been used to describe the gradual decline in GH secretion associated with aging, but pathologic conditions can also cause GH deficiency in adults (Table 1) and must be differentiated from the somatopause.

Table 1. Causes of Growth Hormone Deficiency

Genetic
Transcription factor defects (Pit1, Prop1, Lhx3/4, Hesx1, Pitx2)
GHRH receptor defects
GH secretagogue receptor gene defects
GH gene defects
GH receptor/post receptor defects*
Development anomalies
Septo-optic dysplasia
Holoprosencephaly
Encephalocele
Hydrocephalus
Arachnoid cyst
Midline facial defects
Pituitary hypoplasia
Inflammatory/granulomatous diseases
Encephalitis
Meningitis
Histiocytosis
Sarcoidosis
Autoimmune hypophysitis
Destructive lesions
Intraventricular hemorrhage
Postpartum (Sheehan necrosis)
Cranial radiation
Trauma
Surgery
Hypothalamic/pituitary tumors

Test Application and Interpretation

Laboratory testing remains the most reliable way of diagnosing pathologic GH deficiency. Additionally, laboratory testing should be used to confirm GH deficiency in adults who had isolated GH deficiency in childhood.

GH therapy has substantial positive effects on all the systems affected by GH deficiency and so far appears to be safe in stage IV post marketing trials. It is possible that GH therapy may unmask latent deficiencies in both the thyroid and adrenal axis due to increased deiodination of T4 and increased conversion of cortisol to cortisone. It is thus prudent to monitor these hormones after start of therapy.

Growth Hormone Excess

Growth hormone hypersecretion (acromegaly) has a prevalence of 38 to 69 cases per million population and a yearly incidence of approximately 1000 cases in the United States. Rarely, prepubertal onset occurs, resulting in gigantism. GH hypersecretion is caused by pituitary tumors or, rarely, by extrapituitary GH or GHRH secreting tumors. Approximately 30% of GH secreting pituitary tumors have activating mutations in Gsα. GH hypersecretion is also seen in the McCune Albright syndrome (also due to activating mutations in Gsα), multiple endocrine neoplasia type 1, and the Carney complex. See the section on "Disorders of Anterior Pituitary Function" for a more extensive discussion of GH excess.

Laboratory Assessment of the Growth Hormone–IGF Axis

Table 2 summarizes qualitative laboratory results for disorders of the GH–IGF

Table 2. Laboratory Assessment of the GH–IGF Axis

	GHRH	GH	IGF-I	IGFBP-2	IGFBP-3	BP ratios	GHBP
Classic GH deficiency		↓	↓	↑	↓	↑	N
Partial GH deficiency		N	↓ or N	↑ or N	↓ or N	↑	N
GH resistance/ primary IGF deficiency		↑	↓	↑	↓	↑	↓ N or ↑
Short stature in children due to systemic disease		N	↓ or N	↑ or N	↓ or N	↑ or N	N
Nutritional insufficiency		N or ↑	↓ or N	↑ or N	↓ or N	N	N
Normal short children		N	↓ or N	N	N	N	N or ↓
Adult GH deficiency		N or ↓	↓ or N	↑ or N	↓ or N	↑	N
Acromegaly/ gigantism	N	↑	↑	↓	↑	↓	N
Ectopic GHRH syndrome	↑	↑	↑	↓	↑	↓	N

GHRH, growth hormone releasing hormone; GH, growth hormone; IGF, insulin-like growth factor; IGFBP, IGF binding protein; BP ratios, IGFBP-2/IGF-I ratio and IGFBP-2/IGFBP-3 ratio; GHBP, growth hormone binding protein (circulating, extracellular portion of GH receptor).

Table 3. Characteristics of GH–IGF Axis Hormones and Binding Proteins

Protein	Diurnal Rhythm	Half-Life	Response to	
			GH	IGF-I
GH	Yes	15 min	±	↓↓
IGF-I	No	*	↑↑↑	No
IGF-II	No	*	↑	No
IGFBP-1	No	1-2 h	↑	No
IGFBP-2	No	1-2 h	↓↓	No
IGFBP-3	No	15-20 h	↑↑↑	↑↑
GHBP	No	–	No	No
GHRH	Yes	7 min	No	No

*Half-life of free hormone about 15 min; half-life of bound hormone dependent on binding protein.

axis. Table 3 summarizes characteristics of the hormones for which in vitro assays are available to assess the GH–IGF axis. GH is secreted in a pulsatile pattern and has a short half-life. New, sensitive immunometric assay formats now allow reliable measurement in most subjects. However, single measurements of serum GH are not usually helpful in assessment of GH deficiency, since levels normally are low in normal, awake subjects. Stimulation tests (insulin-induced hypoglycemia, arginine infusion, L-dopa, propanolol, clonidine, or GHRH administration) have been employed with baseline and post-stimulation blood sampling. See "Growth Hormone Dynamic Testing" below.

Insulin-Like Growth Factors and Binding Proteins

Serum IGF-I measurements have become an increasingly useful adjunct in assessment of the GH–IGF axis. IGF-I measurements are useful because its serum level reflects the secretion of GH over several hours. Since IGF-I is protein bound, its half-life in serum is much longer than GH, and it is not subject to pulsatile variability. Low levels of IGF-1 (below the expected sex and age adjusted range) are sufficient to make the diagnosis in subjects with a high pretest likelihood of having GH deficiency such as those with structural lesions and with 3 or more deficient pituitary hormones affected. A normal IGF-1, on the other hand, does not rule out the diagnosis of GH deficiency. Figure 2 shows the pattern of change in IGF-I values vs age for males and females. Data are from 3,961 healthy subjects (1,469 children and adolescents) in Germany, Belgium, and Sweden (Ref. 11). Measurements were made using the Nichols Advantage® assay platform.

The most common acute side effects of GH therapy (arthralgias, edema, carpal tunnel syndrome, irritability) usually can be avoided by individual titration of the GH dose vs clinical response, including side effects, along with normalization of IGF-1 levels to within the age and sex adjusted range. Therefore, therapeutic monitoring with IGF-1 measurements may help optimize therapy.

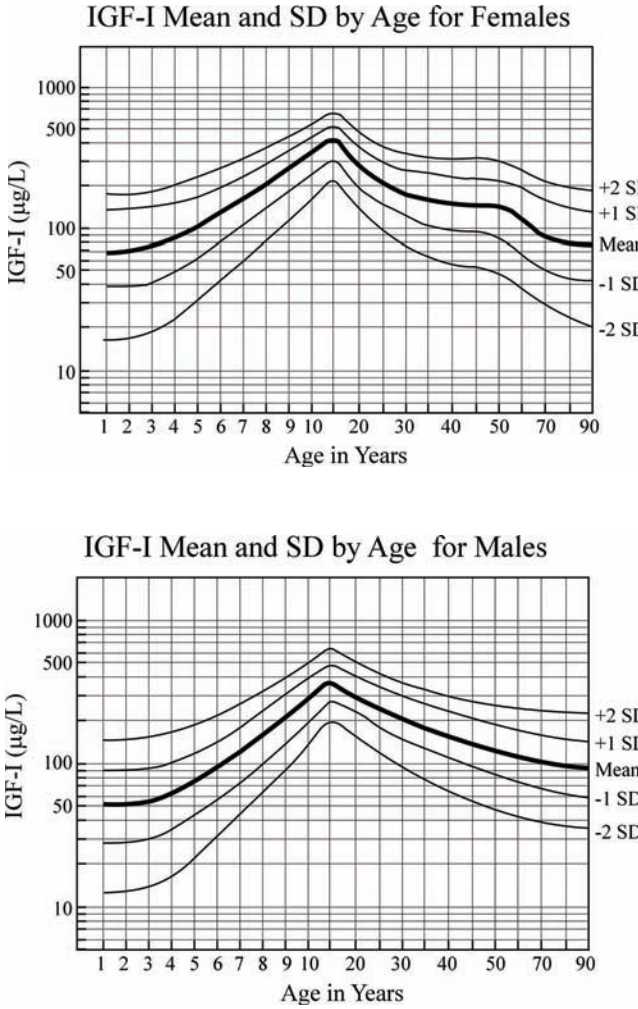

Figure 2. IGF-1 concentrations (mean and standard deviation) stratified by age and gender.

Because GH and IGF-I stimulate IGFBP-3 expression and GH suppresses IGFBP-2 expression, measurement of these binding proteins can also be useful for assessing GH secretory status. It is not clear whether IGFBP-3 has any diagnostic utility in adults, however. Figures 3 to 7 summarize IGF-I, IGFBP-2, IGFBP-3 concentrations and IGFBP-2/IGF-I and IGFBP-2/IGFBP-3 ratio values in children with GH deficiency (GHD) and idiopathic or familial short stature (ISS) relative to 408 controls aged 1 to 19 years.

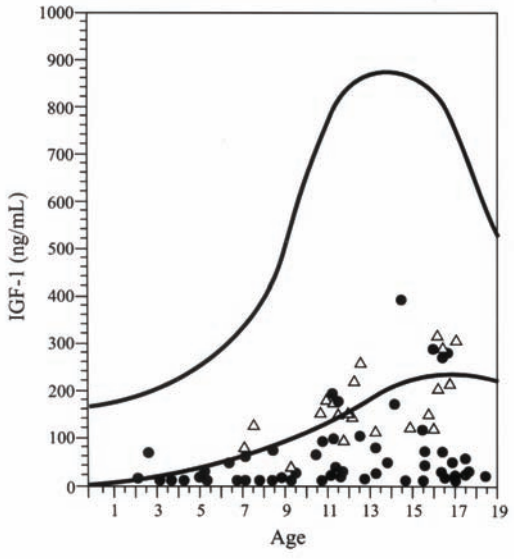

Figure 3. Serum IGF-I concentrations in GHD (solid circles) and ISS (open triangles) children relative to reference range derived from 408 normal children.

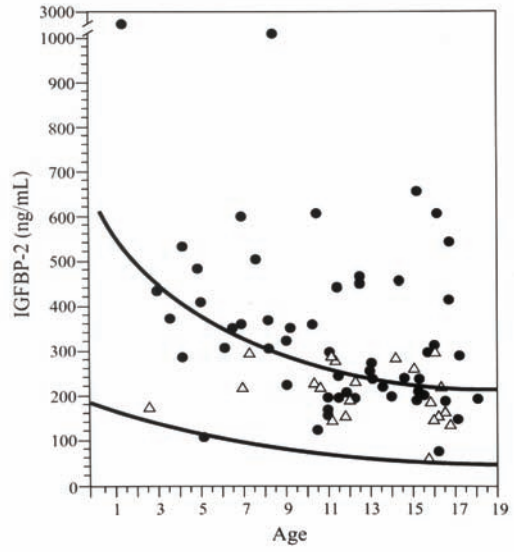

Figure 4. Serum IGFBP-2 concentrations in GHD (solid circles) and ISS (open triangles) children relative to reference range derived from 408 normal children.

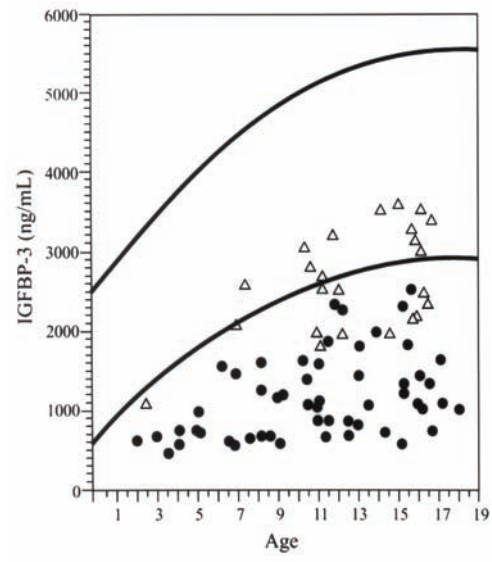

Figure 5. Serum IGFBP-3 concentrations in GHD (solid circles) and ISS (open triangles) children relative to reference range derived from 408 normal children.

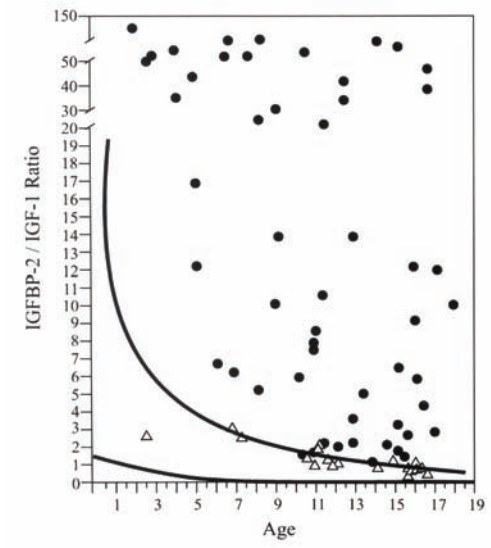

Figure 6. IGFBP-2/IGF-I concentration ratios in GHD (solid circles) and ISS (open triangles) children relative to reference range derived from 408 normal children.

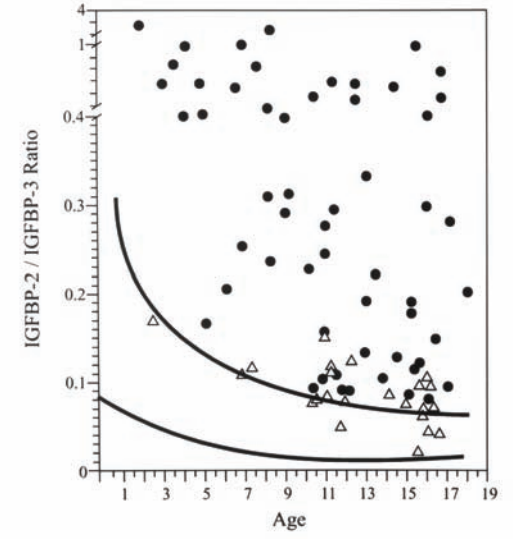

Figure 7. IGFBP-2/IGFBP-3 concentration ratios in GHD (solid circles) and ISS (open triangles) children relative to reference range derived from 408 normal children.

Circulating concentrations of growth hormone binding protein (GHBP) increase with age to maximal levels at puberty. Measurements are useful in the diagnosis of growth hormone resistance and may be useful in predicting responsiveness to exogenous GH in patients being considered for therapy. GH responsiveness tends to vary directly with the GHBP level.

Growth Hormone Dynamic Testing

GH dynamic testing is used for diagnosing both GH excess and deficiency. For the diagnosis of GH excess (acromegaly in adults, gigantism in children), the GH suppression test is employed. This test is basically an oral glucose tolerance test in which the subject receives 75 g of oral glucose, and samples are obtained for GH measurement over the subsequent 30 to 90 min. Patients with acromegaly/gigantism fail to suppress their GH level to <1 ng/mL.

GH stimulation testing has been used for the diagnosis of GH deficiency for several decades. A variety of stimuli have been utilized including physical exercise, insulin-induced hypoglycemia, arginine, clonidine, L-dopa, glucagon, GHRH, and pyridostigmine. Historically, a subnormal GH response to 2 stimuli has been the accepted criterion for diagnosis in children and adults because of the marked variability in responses shown by normal individuals and patients, particularly those with partial GH deficiency. Twenty percent to 30% of normal individuals, however, will fail a given provocative test when using a GH cutoff of 10 ng/mL. Thus, in recent years the use of single tests that simultaneously employ multiple agents (eg, combined GHRH-arginine) has gained favor.

Test Application and Interpretation

The relative performance of various GH stimulation tests in adults has been evaluated, and the diagnostic cut points for the different tests have been established (Ref. 19). This analysis indicated that the insulin tolerance test (ITT) and GHRH-arginine test are the preferred tests for diagnosing GH deficiency in adults. When peak GH cut points of 5.1 ng/mL for the ITT and 4.1 ng/mL for the GHRH-arginine test are employed, these tests show both high sensitivity (96% and 95%, respectively) and specificity (92% and 91%) in diagnosing GH deficiency in this group. Insulin-induced hypoglycemia is contraindicated in patients with a history of seizures or coronary artery disease and may not be necessary in patients with multiple (3 or more) pituitary hormone deficiencies. The GHRH-arginine test is generally well tolerated and free of the potential side effects of hypoglycemia, but because GHRH directly stimulates the pituitary, this test may give a falsely normal response in patients with GH deficiency of hypothalamic origin. In that situation, one of the other tests (for example, arginine alone) should be substituted and the appropriate GH cut point applied.

In children, there is limited data regarding the relative performance of the various GH stimulation tests, and the diagnostic cut points have not been rigorously established. The ITT remains the "gold standard" with an accuracy for predicting GH deficiency ranging from 85% at a GH cutoff of 10 ng/mL to 100% at a cutoff of 3 ng/mL. However, the ITT is not routinely used in children because of the discomfort and potential danger related to hypoglycemia. A recent study examined the reliability of many of the commonly used tests, including the ITT, for assessing GH deficiency (Ref. 9). These investigators concluded that all of the conventional stimuli had high false-positive rates regardless of whether the GH cut point was set at 7 ng/mL or 10 ng/mL. They found that the best tests for distinguishing normal from GH deficient children were GHRH combined with either arginine or pyridostigmine, but, as in adults, these tests may give false-positive results in patients with GH deficiency of hypothalamic origin. In general, the sensitivity (positive predictive value) of the commonly used GH stimulation tests vary from about 40% using a GH cutoff of 10 ng/mL to 80% with a cut off of 2.5 ng/mL. In recent years, most pediatric endocrinologists in North America have adopted a GH cutoff of 10 ng/mL for diagnosing GH deficiency. See GH stimulation tests in the "Dynamic Test Application and Interpretation" section.

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DISORDERS OF THYROID FUNCTION

Background Physiology

The hypothalamic-pituitary-thyroid axis (Figure 1) regulates the production and maintains peripheral concentrations of the biologically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Pituitary thyroid stimulating hormone (TSH) secretion is modulated by the stimulatory effect of hypothalamic thyrotropin releasing hormone (TRH) and the inhibitory (negative feedback) effects of T4 and T3, influencing both TRH and TSH secretion. The major secretory product of the thyroid gland is free T4. It is synthesized as a component of the large (660 kd) precursor thyroglobulin molecule. Iodine is the rate-limiting substrate, which must be actively transported into the thyroid follicular cell by a plasma membrane "iodide pump," a plasma membrane sodium/iodide symporter. A second iodide transporter localized to the apical cell membrane transports intracellular iodide to the luminal cell border where organification occurs, stimulated by thyroid peroxidase. Thyroglobulin, containing the iodotyrosines and the iodinated T4 and T3, is stored as thyroid colloid in the lumen of the thyroid follicles. Iodide trapping, thyroglobulin synthesis, and iodothyronine (T4 and T3) secretion are all stimulated by TSH. The iodothyronines released from thyroid colloid in the process of colloid digestion and iodothyronine secretion are transported in blood bound to the thyroid hormone binding proteins: thyroid hormone binding globulin (TBG) and transthyretin (prealbumin). Thyroid hormones also bind to albumin and lipoproteins with lesser affinity.

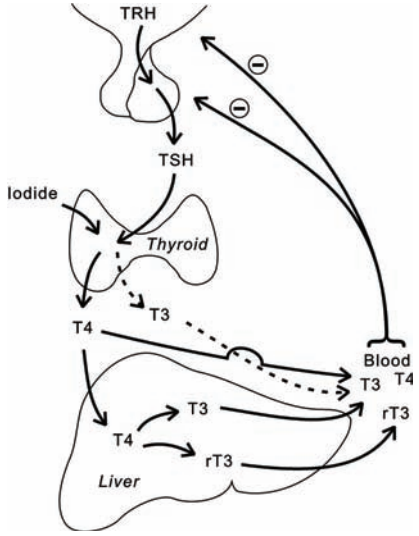

Figure 1. Features of the hypothalamic-pituitary-thyroid axis. TRH, thyrotropin releasing hormone; TSH, thyrotropin; T4, thyroxine; T3, 3,5,3' triiodothyronine; rT3, 3,3,5' triiodothyroxine or reverse T3. See text for details.

Test Application and Interpretation

Only small amounts of T4 and T3 (approximately 0.03% and 0.3%, respectively) are free or unbound; this free hormone is available to tissues. T4 serves largely as a prohormone and is deiodinated in peripheral tissues by several iodothyronine monodeiodinase enzymes to active T3 or biologically inactive reverse T3 (rT3).

The major source of circulating T3 is peripheral conversion from T4, largely by the liver. Only small amounts of T3 are secreted by the thyroid gland in euthyroid subjects ingesting adequate iodine (150 to 300 μg daily). Normally, T4 is deiodinated to both T3 and rT3. The T3 mediates the predominant effects of thyroid hormones via binding to the 50-kd nuclear protein receptors that function as transcription factors modulating thyroid hormone-dependent gene expression. A variety of rapid non-genomic actions of thyroid hormones have been described including changes in solute transport and stimulation of mitochondrial activity and actin polymerization. These actions appear to be triggered by plasma membrane receptors such as MCT8 and aVβ3 integrin.

There are marked variations in thyroid function with age, evident in all of the in vitro hormone and protein measurements. These variations are summarized in Tables 1 and 2. T4 utilization rates decrease progressively with age after birth; other parameters decrease more modestly. In adults, there are no changes with age in serum T4, free T4, or TSH levels, but modest decreases in T3 and T4 utilization are observed with aging. Recent studies show little variation in serum TSH or free T4 concentrations during the hormonal changes of puberty. In general, thyroid function parameters in healthy subjects show little or no clinically significant gender or racial variation. There are a variety of nutritional

Table 1. Changes in Thyroid Function Parameters with Age

Age	Serum Concentrations*				T4 Utilization μg/kg/d
	T4 (μg/dL)	FT4 (ng/dL)	TSH (mU/L)	TBG (mg/dL)	
Fetus					
12-20 wk	0.4-3.9	0-0.4	1-8	0.2-2.3	1.0
21-30 wk	2.7-7.8	0.4-0.9	1.9-8.8	0.8-3.3	2.0
31-40 wk	5.4-14.0	0.9-1.7	3-12	1.5-4.0	5.0
Infant					
1-4 d	14.0-28.4	2.2-5.3	3.2-35	2.2-4.2	10.0
1-4 wk	8.1-15.7	0.9-2.3	1.7-9.1	—	7.0
1-12 mo	5.6-14.9	0.8-2.0	0.8-8.2	1.6-3.6	6.0
Child					
1-5 y	5.6-14.9	1.0-2.4	0.5-4.3	1.2-2.8	5.0
6-10 y	5.6-14.9	1.0-2.4	0.5-4.3	1.2-2.8	4.0
11-15 y	5.6-14.9	1.0-2.4	0.5-4.3	1.4-3.0	3.0
16-20 y	5.6-14.9	1.0-2.4	0.5-4.3	1.4-3.0	2.0
Adult					
21-50 y	5.6-13.7	0.8-2.7	0.4-4.5	1.7-3.6	1.5
51-80 y	5.6-13.7	0.8-2.7	0.4-4.5	1.7-3.6	1.5

T4, thyroxine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TBG, thyroxine-binding globulin. Data from Ref. 2-7.

*Range shown is ±2SD range.

Table 2. Changes in Thyroid Function Parameters with Age

Age	T3 (ng/dL)*	rT3 (ng/dL)*	Thyroglobulin (ng/mL)*	T4 Utilization μg/kg/d
Fetus				
12-20 wk	3.2-25	–	–	1.0
21-30 wk	6.5-49	–	6-230	2.0
31-40 wk	13-97	97-500	2-54	5.0
Infant				
1-4 d	100-740	–	15-101	10.0
1-4 wk	–	26-292	–	7.0
1-12 mo	105-245	11-130	11-92	6.0
Child				
1-5 y	105-269	15-71	5.6-42	5.0
6-10 y	94-241	17-78	5.6-42	4.0
11-15 y	82-213	19-84	2.3-40	3.0
16-20 y	80-210	25-78	2.3-40	2.0
Adult				
21-50 y	71-200	30-78	3.5-56	1.5
51-80 y	39-182	30-78	3.5-56	1.5

T3, triiodothyronine; rT3, reverse triiodothyronine.
Data from Ref. 2-6.
*Range shown is ±2SD range.

and metabolic factors influencing activity of the iodothyronine monodeiodinase enzymes and modulating the rate of peripheral production of T3. The impact of these factors is most clearly manifest in the syndrome of nonthyroidal illness, in which cytokines and/or nutritional deficiencies inhibit T3 production and produce a clinical picture characterized by low levels of T3 with variable T4, reverse T3, and TSH concentrations (the euthyroid sick, low T3, or nonthyroidal illness syndrome). See section on nonthyroidal illness.

Differential Diagnosis of Thyroid Disorders

The spectrum of thyroid function abnormalities with the associated changes in thyroid function test results are summarized in Table 3. The first approach to differential diagnosis of thyroid disorders is the measurement of free T4 and TSH. Free T4 has been assessed indirectly as the product of the total T4 and T3 resin uptake tests but now is usually measured by immunoassay. However, in many disease states, these approaches are misleading. The most accurate assessment of free T4 is by direct dialysis. Figure 2 illustrates a nomogram plotting direct-dialysis free T4 vs third-generation TSH measurements. As illustrated, there is a log-linear relationship between serum TSH and free T4. There is ongoing controversy regarding the upper limit of TSH in euthyroid subjects. Because the negative feedback system is log-linear, a small decrease in free T4

Table 3. Assessment of Common Thyroid Function Disorders

Disorder	TSH	T4	T3	FT4	Tg	TBG	rT3	ATPO	ATG	TBII	TSI	TBA
Primary hypothyroidism	↑	↓	N or ↓	↓	N or ↓	N	↓	n or ↑	n or ↑	n or ↑	n	n or ↑
Transient neonatal hypothyroidism	↑	↓	↓	↓	N or ↓	N	↓	n	n	↑	n	n or ↑
Hashimoto thyroiditis	↑	N or ↓	N or ↓	N or ↓	N or ↓	N	↓	↑	↑	n or ↑	n	n or ↑
Graves disease	↓	↑	↑	↑	↑	N	↑	↑	↑	↑	↑	n or ↑
Neonatal Graves disease	↓	↑	↑	↑	↑	N	↑	n or ↑	n or ↑	↑	↑	n or ↑
TSH deficiency	N or ↓	↓	↓	↓	↓	N	↓	n	n	n	n	n
Thyroid dysgenesis	↑	↓	N or ↓	↓	N or ↓	N	↓	n	n	n	n	n
Thyroid dyshormonogenesis	↑	↓	↓	↓	N ↓ or ↑	N	↓	n	n	n	n	n
Thyroid hormone resistance	N or ↑	↑	↑	↑	↑	N	↑	n	n	n	n	n
TSH dependent hyperthyroidism	↑	↑	↑	↑	↑	N	↑	n	n	n	n	n
T4 protein binding abnormalities	N	V	V	N	N	V*	V	n	n	n	n	n
Nonthyroidal illness	V	N or ↓	↓	V	N	N	N or ↑	n	n	n	n	n
Subacute thyroiditis†	↓ or ↑	↑ or ↓	↑ or ↓	↑ or ↓	↑ or ↓	N	↑ or ↓	n	n	n	n	n

Tg, thyroglobulin; TBG, thyroxine binding globulin; ATPO, antithyroid peroxidase; ATG, antithyroglobulin; TBII, TSH binding inhibiting immunoglobulin; TSI, thyroid stimulating immunoglobulin; TBA, TSH receptor blocking antibody; N, normal; n, negative; V, variable.
*The spectrum of binding protein abnormalities includes increased or decreased TBG binding, increased or decreased transthyretin binding, and ↑ albumin binding.
†Subacute thyroiditis usually involves a transient period of hyperthyroidism followed by a transient hypothyroid state.

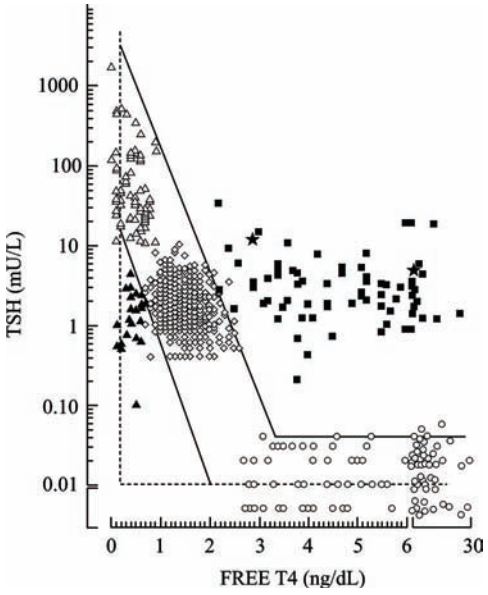

Figure 2. Relationship of serum free T4 concentrations (measured by direct dialysis) and TSH concentration (measured by third-generation assay). Values in normal subjects (ages 2 months to 20 years) and in patients with the indicated thyroid disorders are plotted. ◊, normal; △, primary hypothyroidism; ▲, central hypothyroidism; ○, TSH-independent hyperthyroidism (Graves disease); ■, resistance to thyroid hormone; ★, TSH-secreting pituitary adenoma. The solid lines show the range of values in patients with hypo- or hyperthyroidism.

may be associated with a large increase in serum TSH. This makes TSH the more sensitive determinant for hypothyroidism.

The normal distribution curve for serum TSH is skewed toward higher values. An analysis of serum TSH levels in 17,353 subjects >12 years of age (NHANES III) showed an overall population 97.5 centile upper limit of 5.8 mU/L. Eliminating subjects with self reported thyroid disease and without antithyroid autoantibodies (reference population) lowered the 97.5 centile upper limit to 4.12 mU/L. Median TSH values for the total and reference populations were 1.49 and 1.39 mU/L, respectively (Ref. 7). About 25% of the individuals in NHANES III with TSH levels between 2.5 and 4.5 mU/L had antithyroid antibodies, but there are other reasons for euthyroid subjects having TSH levels in the range of 2.5-4.5 mU/L. These include a shift in the diurnal rhythm such that TSH is higher at the time of phlebotomy, serum leptin elevation in obese subjects, circulatory TSH variants, heterophile antibody, or a modified T4 feedback set point such that the serum TSH output falls in the 2.5-4.5 mU/L range. In about half of the individuals with TSH levels in the 2.5-4.5 mU/L range, there is a slow rate of conversion to overt hypothyroidism. The conversion rate is 2.5% per

year without and about 4.5% per year with detectable thyroid autoantibody. It is recommended that physicians repeat normal range TSH measurements after 1-3 months as clinically applicable and measure anti-TPO antibodies in individuals with levels persistently above 2.5 mU/L. All patients with persistent high normal values can be followed every 1-3 years with repeat serum TSH measurements (Ref. 7).

Blood samples for measurement of TSH concentrations usually are collected randomly. However, there is a modest, but clear, circadian variation in circulating TSH levels in animals and in humans. TSH levels begin to rise several hours before the onset of sleep, and peak levels are observed between 2300 and 0600 hours. Nadir concentrations are observed during the afternoon. The diurnal variation in TSH level approximates ± 50%, so that time of day may have some influence on the measured serum TSH concentration. Daytime TSH levels vary by <10%. The circadian variation in TSH secretion has minimal effect on serum T4 concentrations, however, since the hourly T4 secretion rate (3 to 5 µg) represents a very small fraction of the extrathyroidal T4 pool (500 to 600 µg).

Primary Hypothyroidism

High serum concentrations of TSH indicate thyroid hormone deficiency due to decreased thyroid gland production (primary hypothyroidism). The extent of increase in TSH is, in general, related to the degree of hypothyroidism, but variations above 50 mU/L have limited significance in this regard. Mild, compensated hypothyroidism (referred to as subclinical hypothyroidism) may be difficult to diagnose. A modest reduction in total and free T4 within the normal range, with a normal level of serum T3, may be associated with a serum TSH level in the 5 to 10 mU/L range. Some physicians treat and others observe such patients depending upon the clinical circumstances, serum cholesterol, and whether antithyroid antibodies are present. In general, serum TSH concentrations above 10 mU/L in patients with primary thyroid disease are treated.

Hyperthyroidism

Decreased levels of TSH in patients with a normally functioning hypothalamic pituitary axis imply increased thyroid hormone secretion or release by the thyroid gland, suppression by exogenous thyroid hormone, or an ectopic source of thyroid hormone, such as struma ovarii. The most common cause of hyperthyroidism is Graves disease, which is due to TSH receptor stimulation by the thyroid-stimulating immunoglobulin (TSI) autoantibody. Human chorionic gonadotropin has weak TSH-like bioactivity and can produce hyperthyroidism when greatly elevated in pregnant patients with choriocarcinoma or hydatidiform mole. Hyper-functioning autonomous thyroid nodules may secrete enough thyroid hormone to produce hyperthyroidism. Subacute (probably viral) thyroiditis, sometimes referred to as subacute painful thyroiditis, classically is characterized by a transient (several week) period of hyperthyroidism, with TSH suppression, followed by a transient (several week to several month) period of hypothyroidism with TSH elevation. Moderate or severe hyperthyroidism usually suppresses serum TSH levels to 0.03 mU/L or less. However, milder sub-clinical disease or overzealous thyroxine therapy may result in levels in the 0.03 to 0.3 mU/L range. After treatment of hyperthyroidism, serum TSH may remain

suppressed for several weeks despite normalization or reduction of free T4 and free T3 concentrations before normal TSH regulation resumes. In treatment of both hyper- and hypothyroid patients, repeat measurement of TSH should be done after a 6-week interval. This is the time required for TSH to achieve a steady state after a change in therapeutic dose or regimen.

Hypothalamic-Pituitary Hypothyroidism

Hypothalamic TRH deficiency or pituitary TSH deficiency from any cause is associated with thyroid gland hypofunction. Hypothalamic or pituitary anomalies, neoplastic or inflammatory diseases involving the hypothalamic-pituitary axis, or congenital abnormalities of TRH or TSH secretion may be involved. These disorders have been referred to as secondary (pituitary) or tertiary (hypothalamic) hypothyroidism. Serum TSH levels are in the low or normal range in the presence of low concentrations of free T4. TSH bioactivity tends to be reduced in these patients because of abnormal glycosylation of the secreted and circulating TSH isoforms. Pituitary TSH deficiency can be confirmed by showing a deficient TSH response to exogenous TRH. Patients with hypothalamic TRH deficiency and a normal pituitary gland usually manifest a normal TSH response to TRH, but the response may be relatively prolonged, persisting for 30 to 60 minutes rather than falling off after 30 minutes. Patients with these central forms of hypothyroidism plot below or to the left of the normal range, as shown in Figure 2.

Inappropriate TSH Secretion

Patients with inappropriate TSH secretion manifest increased free T4 and free T3 levels without suppression of serum TSH concentrations. Increased TSH levels may be due to thyroid hormone resistance or to a TSH-secreting pituitary tumor. Thyroid hormone resistance has been shown to be due to a variety of mutations in the beta thyroid hormone receptor gene, which variably impairs the ability of the receptor proteins to activate transcription of target genes. The tissue distribution of the abnormal receptor, vs the normal thyroid hormone receptor alpha, appears to determine the phenotype of the patient in terms of thyroid function. Patients may appear near normal or may manifest variable signs and symptoms of hypo- or hyperthyroidism. Patients with generalized thyroid hormone resistance involving the pituitary and peripheral tissues have normal or elevated TSH and elevated free T4 levels, with or without clinical manifestations of hypothyroidism. Patients with isolated pituitary resistance have similar thyroid laboratory test results but present with clinical thyrotoxicosis of variable degree. Adenomas involving pituitary thyrotroph cells are rare but can produce inappropriate secretion of TSH with associated hyperthyroidism. Patients with inappropriate TSH secretion plot above or to the right of either normal individuals or thyroid patients with intact hypothalamic-pituitary function, as shown in Figure 2. See also the "Disorders of Anterior Pituitary Function" section.

Nonthyroidal Illness

This syndrome, also referred to as the low T3 or euthyroid sick syndrome, is characterized by low total and free serum T3; normal, low, or high total T4; increased T4 sulfate; normal to high free T4; and normal TSH concentrations. It

has been reported in a variety of clinical situations including premature infants and patients with a variety of severe acute and chronic illnesses. The latter include diabetic ketoacidosis, severe trauma, burns, febrile states, cirrhosis, and renal failure. A similar syndrome may be produced by a number of drugs including dexamethasone, selected radiographic contrast agents, propylthiouracil, propranolol, and amiodarone. Three patterns of change in thyroid hormone levels have been described (Table 4). The low serum and tissue T3 levels in these patients occur as a result of inhibition of iodothyronine β-ring monodeiodinase-1 activity (MDI-1) and a decreased rate of T3 production from T4 in non-thyroidal tissues. In mice, interleukin-1 (IL-1) cytokine inhibition of iodothyronine monodeiodinase type 1 (MDI-1) transcription results in decreased hepatic T4 to T3 conversion, and this reduction can be reversed by steroid receptor coactivator-1 (SRC-1) induction of MDI-1. rT3 is increased because the degradation of rT3 is mediated by the same type 1 deiodinase enzyme that mediates T4 to T3 conversion. Thus, serum T3 levels fall, and rT3 levels tend to remain normal or increase. In some patients, serum T4 levels also fall (the low T4 syndrome), and low T4 levels in patients with severe nonthyroidal illness have been associated with increased mortality. TBG levels may be reduced in such patients, and an inhibitor of T4 binding to TBG has been described in the serum and derived from the tissues of such patients. The high T4 nonthyroidal illness syndrome probably involves one or more induced abnormalities in the disposal pathways of T4. In some patients, such as those receiving amiodarone or those with psychiatric illness, free T4 may be elevated with or without a decreased serum TSH. A suppression of TSH during the acute phase of the psychiatric illness may be associated with a transient rebound of TSH above the normal range followed with normalization thereafter. There is no convincing evidence that treatment with thyroid hormones is beneficial in most patients with nonthyroidal illness, but in selected clinical conditions, such as postoperative cardiac surgery, treatment has been advocated.

Autoimmune Thyroid Disease

The most frequently encountered thyroid disorders are autoimmune in nature. It is now clear that both cellular- and antibody-mediated pathogenetic mechanisms are involved and that the autoimmune diathesis in affected patients has a familial, genetic predisposition. Table 5 summarizes the clinical spectrum of autoimmune thyroid disease. The most common disorders are Hashimoto chronic (lymphocytic) thyroiditis (HT) and Graves disease (GD). HT involves both cell- and antibody-mediated destruction of thyroid tissue, but the cell-mediated component is the most important. In patients with GD, by contrast, the hyperthyroid state is caused by TSH receptor-stimulating autoantibodies mediating a TSH-like effect and thyroid cell hyperplasia.

Table 4. Thyroid Function Abnormalities in Nonthyroidal Illness

NTI Pattern	TSH	T3	rT3	T4
Low T3 syndrome	N	↓	N or ↑	N
Low T4 syndrome	N	↓	N or ↑	↓
High T4 syndrome	N	↓	N or ↑	↑

NTI, nonthyroidal illness; rT3, reverse T3; N, normal, ↓, decreased; ↑, increased.

Table 5. Clinical Spectrum of Autoimmune Thyroid Disease

Thyroid disorders
Hashimoto thyroiditis
Primary myxedema
Fibrous thyroiditis
Thyroid lymphoma
Hashitoxicosis
Atrophic thyroiditis
Graves disease
Autoimmune polyglandular disease, type II*
Hashimoto thyroiditis
Diabetes mellitus
Addison disease
Associated autoimmune disorders
Vitiligo
Pernicious anemia
Idiopathic thrombocytopenia
Thyroid antigen/antibody nephritis
Pregnancy-associated thyroid disorders
Transient postpartum hypothyroidism
Transient postpartum hyperthyroidism
Neonatal Graves disease
Neonatal hypothyroidism

*May include ovarian failure, pernicious anemia, myasthenia gravis, vitiligo, and alopecia.

A variety of antibody types have been associated with thyroid autoimmune diseases (Table 6). The classic thyroid autoantibodies are directed against thyroglobulin (ATG), the thyroid hormone precursor and storage protein in the thyroid gland, and against thyroid microsomal membrane antigens, the most important being thyroid peroxidase (ATPO). Other prominent antibodies are directed against the TSH receptor. Both receptor-stimulating immunoglobulins (TSI) and TSH receptor blocking antibodies (TBA) have been characterized. TSIs are identified by their capacity to stimulate cyclic AMP (cAMP) production in thyroid follicular cells in culture; TBA blocks TSH-stimulated cAMP production. Both TSI and TBA will displace labeled TSH from partially purified TSH receptors in a membrane radioreceptor assay system. In this instance, the antibody is referred to as TSH binding inhibitory immunoglobulin (TBII). A collagen-stimulating immunoglobulin (CSI) has been characterized that stimulates incorporation of labeled proline into collagen by fibroblasts in tissue culture. A thyroid growth-stimulating immunoglobulin (TGI) has been described that stimulates incorporation of tritiated thymidine into DNA of thyroid follicular cells in culture and promotes thyroid cell replication. The CSI and TGI autoantibodies have not been utilized clinically.

Table 6. Thyroid Autoantibodies in Human Subjects

ATG	Thyroglobulin antibody
ATPO	Thyroid peroxidase antibody
Anti-T3	Triiodothyronine antibody
Anti-T4	Thyroxine antibody
Anti-TSH	Thyroid stimulating hormone antibody
TSH Receptor Antibodies	
TSI	Thyroid stimulating immunoglobulin
TBII	TSH binding-inhibitory immunoglobulin
TBA	TSH blocking antibody
Other Antibodies	
CSI	Collagen stimulating immunoglobulin
TGI	Thyroid growth stimulating immunoglobulin

Application of clinically useful autoantibody assays to the differential diagnosis of autoimmune disease is summarized in Table 7. The ATG and ATPO autoantibodies are markers for thyroid autoimmune diathesis and may be detected in autoimmune disease family members who have not yet developed thyroid disease. Antibody titers are not usually indicative of disease severity. Occasional patients with autoimmune disease may also manifest autoantibodies to T4, T3, or TSH, and such antibodies may interfere with immunoassay measurements for these hormones.

Table 7. Qualitative Thyroid Autoantibody Test Results in Various Thyroid Autoimmune Disease States

Type	ATG	ATPO	TSI	TBII
Hashimoto's thyroiditis				
Juvenile	+	+		+
Adult	+++	+++		+
Hashitoxicosis	++	++	++	++
Atrophic thyroiditis	+	+		++
Graves disease				
Juvenile	+	+	++	++
Adult	+	+	+++	+++
With opthalomopathy	+	+	+++	+++
With dermopathy	+	+	+++	+++
Transient neonatal hypothyroidism	+	+		++
Neonatal Graves disease	+	+	+++	+++

ATG, antithyroglobulin antibody; ATPO, antithyroid peroxidase antibody; TSI, thyroid stimulating immunoglobulin; TBII, TSH binding inhibitory immunoglobulin.

Test Application and Interpretation

There appears to be significant autoantibody heterogeneity in most patients with autoimmune disease, and although ATPO is present in most patients with Hashimoto thyroiditis and TSI in most patients with Graves disease (about 90% and 70%, respectively), adding the ATG for Hashimoto disease and adding TBII and TGI for Graves disease increases test positivity to 95% and 90%, respectively.

Thyroid autoimmune disease may be associated with autoimmune syndromes involving other endocrine glands and other tissues (Table 5). The most common association is with autoimmune insulin-dependent diabetes mellitus. The triad of Hashimoto thyroiditis, diabetes mellitus, and adrenal insufficiency has been referred to as autoimmune polyglandular disease type II, and this syndrome also can include ovarian atrophy, testicular failure, lymphocytic hypophysitis, pernicious anemia, myasthenia gravis, vitiligo, and alopecia. Autoimmune polyendocrinopathy type I with candidiasis and ectodermal dystrophy (APECED) is usually associated with hypoparathyroidism, adrenal failure, diabetes mellitus, pernicious anemia, vitiligo, and alopecia but does not usually include autoimmune thyroiditis. This rare syndrome is autosomal recessive and is frequently associated with mutations in the *AIRE* (autoimmune regulator) gene. In contrast, type II polyglandular autoimmune disease is relatively common. In patients with insulin-dependent diabetes mellitus, the prevalence of elevated serum TSH levels approximates 20% in female patients and 5% in males at all ages.

Associated autoimmune disorders in patients with autoimmune thyroid disease also can include idiopathic thrombocytopenia and thyroid antigen/antibody nephritis (Table 5). Patients may have platelet antibodies with or without clinical manifestations of thrombocytopenia. Circulating immune complexes also are present in some patients with autoimmune thyroid disease, and several patients have been reported with thyroglobulin and/or microsomal antigens identifiable by immunohistochemistry in the glomeruli as part of the kidney fixed antigen-antibody complex.

Pregnancy tends to ameliorate the manifestations of autoimmune thyroid disease. This presumably is due to the mechanism(s) operative during pregnancy to protect the fetal allograft from rejection. This improvement in thyroid function during pregnancy is followed in many who are not taking medication by a return or exacerbation of clinical disease during the postpartum period. This usually is transient and may resemble Hashimoto thyroiditis or Graves disease. Neonatal hypothyroidism or Graves disease also may be observed. In women developing postpartum thyroiditis, the thyroid autoantibody titers increase to peak levels 4 to 7 months postpartum in parallel with the changes in free T4 and TSH concentrations.

A recently described syndrome referred to as silent thyroiditis, or painless thyroiditis with transient hyperthyroidism, resembles subacute thyroiditis in that transient thyrotoxicosis occurs in association with a low thyroid radioiodine uptake. However, these patients occasionally have low titers of thyroid autoantibodies. The thyroid histology in this condition shows a lymphocytic infiltrate. This postpartum hyperthyroid state also occurs in nonpregnant persons, but the disorder is more commonly seen in the postpartum period.

Acute thyroid cell cytotoxicity presumably accounts for the transient hyperthyroidism and low radioiodine uptake.

Thyroid Dysfunction in Infancy and Early Childhood

Most infants with congenital hypothyroidism are detected in newborn thyroid-screening programs. The prevalence approximates 1 in 4,000 infants. The majority manifest primary hypothyroidism due to thyroid gland agenesis or dysgenesis. Ten percent to 15% of cases are due to a congenital defect in TSH action or a biosynthetic defect in thyroid gland hormonogenesis. In another 10%, the hypothyroidism is transient because of antithyroid drugs or maternal TSH receptor-blocking antibodies. Some 3% to 5% (about 1 in 30,000 newborns) manifest hypothalamic-pituitary TSH deficiency. One percent to 3% represents familial cases of thyroid dysgenesis associated with mutations of the thyroid transcription factors (TTF-1, *NKF2*.1; TTF-2, *FOXE1*), or the *PAX-8* genes, which are involved in thyroid gland embryogenesis.

Neonatal hypothyroidism due to maternal TSH receptor blocking antibody usually occurs in association with maternal atrophic thyroiditis. Patients with atrophic thyroiditis have elevated titers of TSH receptor blocking antibody (TBA), which blocks TSH stimulation of thyroid gland function. Thyroid radioiodine uptake is low, and thyroid hormone secretion is reduced. Serum TSH levels are elevated, and the thyroid gland is hypoplastic or atrophic. ATPO and ATG titers may be elevated. The autoantibody levels in maternal and neonatal blood usually are similar, so that measurement in the mother can avoid newborn sampling. The TBII and/or TBA tests will detect TSH receptor blocking antibodies. A number of genetic abnormalities in the pituitary-thyroid axis producing neonatal thyroid dysfunction have been described (Table 8).

Laboratory tests for the assessment of thyroid disorders in the neonatal period are summarized in Table 9. Absent or very low levels of serum thyroglobulin supports a diagnosis of thyroid agenesis. The presence of TSH receptor blocking antibody in maternal or newborn serum, assessed as TSH binding inhibiting immunoglobulin (TBII) or TBA, supports a diagnosis of transient hypothyroidism. TBA or an inactivating TSH receptor gene mutation can lead to an erroneous diagnosis of thyroid agenesis by thyroid scan. Ultrasound imaging is more reliable. Some physicians prefer to delay the differential diagnostic assessment of congenital thyroid disease to 2-3 years of age to assure early and adequate thyroxine replacement therapy.

Thyroid Neoplasia

A classification of primary thyroid neoplasms is shown in Table 10. These tumors typically present as single thyroid nodules. Diagnosis usually involves thyroid function testing and fine needle aspiration/cytology with or without radioiodine or ultrasound scanning. In the diagnosis and/or management of thyroid neoplasia, 2 thyroid proteins serve as important tumor markers: calcitonin and thyroglobulin. Calcitonin is a 32-amino acid polypeptide hormone produced exclusively by parafollicular or C-cells in the thyroid. The hormone inhibits osteoclast mediated bone resorption, but its physiological role is uncertain. The

Table 8. Genetic Abnormalities of Thyroid Hormone Metabolism*

Abnormalities	Prevalence	Molecular Abnormality
Hypothalamic-pituitary defects	1:20,000-30,000	*SHH*, *ZIC2*, *SIX3*, *HESX1*, *LHX3*, *LHX4*, *PROP1*, *PIT1*, *POUF1* mutations
Familial TSH deficiency	Rare	TSH/β gene mutations
TSH unresponsiveness	Rare	TSH receptor gene mutations
Iodide transport defect	Rare	Sodium/iodide symporter (*NIS*) gene mutations
Pendred syndrome	1:50,000	Chloride/iodide transporter (Pendrin gene) mutations
Organification defects	1:40,000	Thyroid peroxidase gene mutations; defective H_20_2 generation (*THOX* mutations)
Thyroglobulin defects	1:40,000	Thyroglobulin gene mutations; sialyltransferase defect
Iodotyrosine deiodinase defects	Rare	Iodotyrosine deiodinase gene (*DHALI*) mutations
Iodothyronine deiodinase deficiency	Rare	*SECISBP2* gene mutations
Thyroid hormone transporter defect associated with X-linked paroxysmal dyskinesia	Rare	Monocarboxylate transporter (*MCT8*) gene mutations
Thyroid hormone resistance	1:100,000	Thyroid nuclear receptor (TRbeta) gene mutations
Autosomal dominant hyperthyroidism	Rare	Activating TSH receptor gene mutations

*All produce hypothyroidism of variable degree except for the activating TSH receptor mutations producing hyperthyroidism.

calcitonin measurement is indicated for the diagnosis and follow-up of patients with medullary thyroid carcinoma (MTC), the majority of whom produce the hormone. Settings in which the test is appropriate include 1) thyroid nodule patients; 2) screening of individuals in families with known or suspected MTC or the multiple endocrine neoplasia syndrome types 2A (MTC, hyperparathyroidism, pheochromocytoma) or 2B (MTC, pheochromocytoma, submucosal neuromas, and Marfanoid body habitus); and 3) follow-up of patients with known MTC. Screening MTC family members with normal basal calcitonin levels should usually include additional calcitonin measurements after calcium stimulation (See "Dynamic Test Application and Interpretation" section). The carcinoembryonic antigen (CEA) may also be a useful tumor marker in certain MTC patients.

Table 9. Laboratory Tests for the Diagnosis of Neonatal Thyroid Disorders

Disease	In Vitro Tests								In Vivo Tests			
	TT4	FT4	TSH	TT3	Tg	TBII	TBA	TSI	Scan Uptake	Perchlorate Discharge	Ultrasound	TRH Response TSH	TRH Response TT4
Thyroid dysgenesis	N or ↓	N or ↓	↑	N or ↓	O to ↑				A		A		
Thyroid dyshormonogenesis	N or ↓	N or ↓	↑	N or ↓	N or ↑				N or A	N or A	N		
Iodide symporter defect	N or ↓	N or ↓	↑	N or ↓	N or ↑				A		N		
TSH receptor mutation													
Inactivating	N or ↓	N or ↓	↑	N or ↓	N or ↓	n	n	n	N or A		N		
Activating	↑	↑	↓	↑	↑	n	n	n	N		N		
Hypothalamic defect	↓	↓	N or ↓	↓	↓				N		N	N	N or ↓
Pituitary defect	↓	↓	N or ↓	↓	↓				N		N	↓	↓
Transient hypothyroidism													
TSH receptor antibody	↓	↓	↑	↓	↓	↑	↑	n	N or A		N		
PTU/MMI/I toxicity	↓	↓	↑	↓	↓	n	n	n	N	N	N		
Transient hyperthyroidism (neonatal Graves disease)	↑	↑	↓	↑	↑	↑	rare ↑		N		N		
Nonthyroidal illness	N or ↓	N or ↑	N	↓	N or ↓	n	n	n	N		N		
Premature													
Hypothyroxinemia	↓	↓	N	↓	↑				N		N		
Transient 1° hypothyroidism	↓	↓	↑	↓	↑	n	n	n	N		N	↑	N or ↓
Transient 3° hypothyroidism	↓	↓	N or ↓	↓	N or ↓				N		N	N	N or ↓

Tg, thyroglobulin; TBII, TSH binding inhibiting immunoglobulin; TSI, thyroid stimulating immunoglobulin; O, undetectable; N, normal; n, negative; A, abnormal; PTU, propylthiouracil; MMI, methimazole; I, iodine.

Table 10. Classification of Thyroid Neoplasia

Thyroid adenoma
Colloid
Embryonal
Follicular*
Hürthle cell tumor
Thyroid carcinoma
Papillary
Follicular
Hürthle cell
Medullary
Undifferentiated
Thyroid lymphoma

*Occasionally functioning or toxic adenoma.

It is now clear that familial MTC and most cases of sporadic MTC are caused by mutations in the *RET* protooncogene. Definitive diagnosis is now possible by mutation analysis and early *RET* genotyping of young children in known kindreds, followed by prophylactic thyroidectomy of affected patients (see "Pheochromocytoma, Medullary Thyroid Carcinoma, and Multiple Endocrine Neoplasia").

Thyroglobulin (Tg) is a 660-kd protein synthesized exclusively by the thyroid follicular cells. Tg serves as the prohormone for iodothyronines and normally is largely sequestered within the thyroid gland. However, small amounts are released in the process of Tg synthesis. Circulating Tg levels are increased in all forms of endogenous hyperthyroidism, including Graves disease, toxic nodular goiter, and subacute thyroiditis. Levels also are increased in many patients with thyroid adenoma and most patients with papillary/follicular carcinoma. Thus, Tg measurements cannot distinguish benign and malignant thyroid nodules, but serial measurements are very valuable in the postoperative monitoring of patients with papillary, follicular, and Hürthle cell carcinomas. The test is more sensitive for detection of residual or recurrent disease when serum samples are obtained after withdrawal of thyroid suppression therapy, when the patients' endogenous TSH concentration is high, or after administration of recombinant TSH. The peak serum Tg after recombinant human TSH (rhTSH) usually is seen 3 days after a second injection. Measurement of serum Tg currently is the most sensitive marker for thyroid cancer recurrence. Most patients with undetectable levels after initial surgery and without replacement thyroxine remain free of relapse after 15 years follow-up. Sixty percent to 80% of patients requiring thyroxine replacement and with serum Tg levels >10 ng/mL after thyroxine withdrawal had local recurrence or distant metastases as assessed by large dose radioiodine scanning. Twenty percent to 30% of patients with thyroid carcinoma have circulating ATG, which renders Tg measurements unreliable.

Test Application and Interpretation

Thyroid Protein Binding Abnormalities

Laboratory differentiation of patients with euthyroid hyperthyroxinemia from those with hyperthyroidism is sometimes difficult, especially if the hyperthyroidism is pituitary in origin. Euthyroid hyperthyroxinemia may result from an increase in the concentration of thyroxine-binding globulin (TBG), increased concentrations of transthyretin (prealbumin), or the presence of molecular variants of transthyretin or of albumin with increased affinity for T4. The last-mentioned disorder is referred to as familial dysalbuminemic hyperthyroxinemia (FDH). Distinguishing euthyroid hypothyroxinemia from hypothyroidism, particularly from secondary and tertiary hypothyroidism, is also difficult. Euthyroid hypothyroxinemia can result from a decreased TBG concentration, the presence of molecular variants of TBG with reduced T4-binding affinity, or the euthyroid hypothyroxinemia of illness (the low-T4 state of nonthyroidal illness). The TBG gene is localized to the X chromosome, and male hemizygotes with a single mutant allele can show one of 3 phenotypes: absent, decreased, or increased TBG and bound T4 values. There are more than 15 known X-linked TBG mutants: some cause complete TBG deficiency, and others cause reduced affinity for T4 binding. The prevalence of TBG deficiency in males approximates 1:5000 with 1:15,000 showing complete deficiency. Thus TBG deficiency is as prevalent as congenital hypothyroidism and may contribute to confusion in diagnosis of congenital hypothyroidism in screening programs. The prevalence of TBG excess approximates 1:25,000 newborn males.

Some clues to the T4 protein binding disorder can be obtained by assessing serum T4 and T3 levels. Increased TBG binding is associated with increased levels of both T4 and T3, since TBG binds both hormones. Increased transthyretin levels are associated with increased T4 but normal T3 concentrations, since this protein preferentially binds T4. Free T4 concentration, measured by direct dialysis, is normal in these patients. However, an accurate differential diagnosis requires measurement of the protein level and/or the distribution of T4 among the binding proteins in association with the concentration of protein-bound T4 per unit mass of the respective protein. This is accomplished via gel electrophoresis of radioiodinated thyroxine-labeled serum samples; measurement of T4, TBG, prealbumin (transthyretin), and albumin concentrations; and determination of the distribution of labeled T4 among the proteins of the electrophoretogram. See "T4 Binding Proteins" in the "Alphabetical Test" section for results of such analysis in normal subjects. Relative to the normal values, patients with abnormal protein concentrations and abnormal protein binding usually can be distinguished.

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Test Application and Interpretation

FLUID AND ELECTROLYTE DISORDERS

Background Physiology

Body salt and water metabolism are largely regulated by aldosterone, arginine vasopressin (AVP, antidiuretic hormone, ADH), and atrial natriuretic hormone (ANH), which control the renal excretion of sodium and free water (Figure 1). Aldosterone production is controlled via the renin-angiotensin system, which in turn is modulated by renal blood flow as a parameter of body water and blood volume. AVP secretion is modulated primarily by plasma osmolality (increased osmolality increases and decreased osmolality decreases AVP secretion) via hypothalamic osmoreceptors and secondarily by cardiac atrial volume receptors (increased volume decreases and decreased volume increases AVP release). Aldosterone acts to increase renal sodium retention by increasing potassium excretion in exchange for reabsorbed sodium. AVP acts on the kidney collecting tubule to reabsorb free water and increase urine osmolality.

One additional control factor is the natriuretic hormones (eg, ANH, also called atrial natriuretic peptide [ANP], and B-type natriuretic peptide [BNP]) secreted by cardiac atrial tissue in response to changes in atrial volume (increased volume/pressure increases and decreased volume decreases ANH

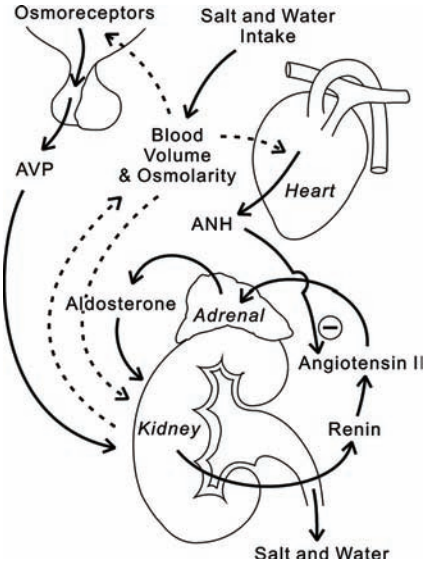

Figure 1. The regulation of blood volume and osmolality by arginine vasopressin (AVP), aldosterone, and atrial natriuretic hormones (ANH). See text for details.

secretion (Table 1). ANH acts to inhibit the renin-angiotensin system, and inhibiting endogenous ANH degradation by blocking endopeptidase leads to significant natriuresis and renin-angiotensin suppression. ANH has been proposed as the mechanism for the "escape phenomenon," which limits the extent of hyponatremia and blood volume expansion in primary hyperaldosteronism and the syndrome of inappropriate antidiuretic hormone secretion (SIADH). The assay for serum BNP is now utilized as a marker for congestive heart failure (CHF), increasing levels correlating with increasing severity of CHF. A third natriuretic peptide, C-type natriuretic peptide (CNP), has been characterized but has no clinical diagnostic application to date.

The endothelins (ET1, ET2, and E3) and adrenomedullin also have been shown to have significant roles in cardiovascular renal physiology (Table 1). The circulating levels are low (<10 pmol/L) and, in the case of the endothelins, blood levels are increased in a variety of disease states. Adrenomedullin levels are elevated in dialysis patients, in ischemic stroke, and in women with recurrent pregnancy loss. However, a clinical diagnostic application for either peptide remains to be defined.

Primary Disorders

Laboratory features of the primary disorders of aldosterone or AVP secretion/metabolism are summarized in Table 2. Laboratory testing plays a vital role in the evaluation of patients with nonglycosuric polyuria, in whom neurogenic or central

Table 1. Physiological Effects of the Atrial Natriuretic Peptides, Endothelin 1, and Adrenomedullin

	ANP/BNP	CNP	Endothelin 1	Adrenomedulin
Site of synthesis	Heart	Brain	Vascular endothelium	Vascular endothelium
Secretion stimulus	Transmural pressure	Local factors	Local factors	Local factors
Plasma half-life	3-22 min	2-3 min	1-2 min	20 min
Receptor	NPR-A	NPR-B	ETA, ETB	HM-R, CGAP-R, RDC-1
Intracellular mediator	cGMP	cGMP	Ca, NO	cAMP, NO
Vascular effect	Dilation	Dilation	Constriction/dilation	Dilation
Cardiac effect	↑ coronary flow	↑ coronary flow	↓ coronary flow ↑ ANP/BNP	↑ coronary flow
Kidney	Natriuresis ↓ Renin	—	Antinatriuresis ↓ Renin	Natriuresis ↓ Renin
Adrenal	↓ Aldosterone	—	↑ Aldosterone	↓ Aldosterone

From Redemaker and Espiner, Ref. 3.

Table 2. Laboratory Features of Primary Disorders of Salt and Water Metabolism

Disorder	Serum Na +	Serum K +	Serum Aldo	Urine Aldo	Urine Na +	Plasma OSM	Plasma AVP	Urine OSM	Urine AVP	Plasma ANH	Plasma Renin*	DNA Analysis
Diabetes insipidus	↑	N	N	N	N	↑	↓	↓	↓	—	N	Yes†
SIADH	↓	N	N	N	N	↓	↑‡	↑	↑‡	↑	N	
Nephrogenic diabetes insipidus (AVP resistance)	↑	N	N	N	N	↑	↑	↓	↑	—	N	Yes†
Psychogenic polydipsia	↓	N	N	N	N	↓	↓	↓	↓		N	
Primary aldosteronism	N	↓	↑	↑	N–↓	N	N	N	N	↑	↓	
Hypoaldosteronism	↓	↑	↓	↓	↑	N	N	N	N	—	↑	
Hyporeninemic hypoaldosteronism	N	↑	↓	↓	↑	N	N	N	N	—	N or ↓	
Pseudohypoaldosteronism	↓	↑	↑	↑	↑	N	N	N	N	—	↑	

Aldo, aldosterone; OSM, osmolality; AVP, arginine vasopressin; ANH, atrial natriuretic hormone; SIADH, syndrome of inappropriate vasopressin (ADH) secretion.
*Plasma renin, direct renin or plasma renin activity (PRA).
†For genetic mutations causing central or nephrogenic diabetes insipidus.
‡Increased relative to plasma osmolality but normal relative to urine osmolality.

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diabetes insipidus, nephrogenic diabetes insipidus, and psychogenic polydipsia must be differentiated. In addition to basal serum and urine osmolalities, the plasma vasopressin assay (Figures 2 and 3) and water deprivation and vasopressin response tests may be required (see "Dynamic Test Application and Interpretation" section). DNA analysis now is available to characterize the neurophysin and aquaporin gene mutations associated with central and nephrogenic diabetes insipidus. Laboratory testing for disorders of aldosterone metabolism are reviewed in the "Disorders of Adrenal Function" section.

Secondary Disorders

The common disorders of fluid and electrolyte metabolism are those involving secondary changes in aldosterone and AVP production. These include congestive heart failure, cirrhosis and ascites, the nephrotic syndrome, salt loss syndromes (diarrhea, diuretic therapy, renal salt-losing disorders), severe hypothyroidism, and adrenal insufficiency. In most such patients, the diagnosis can be established on the basis of clinical observation and more routine laboratory assessments (serum sodium, liver function tests, serum creatinine and BUN, urine sodium and osmolality). In most instances, these disorders are associated with hyponatremia. They can be divided into hypervolemic, euvolemic, and hypovolemic states, as indicated in Table 3.

Hyponatremia may be factitious in patients with marked elevations of plasma lipids or proteins because of a relative decrease in the aqueous fraction of plasma. This situation is likely if there is an associated elevation of the serum triglyceride or total protein concentration and is confirmed by finding a normal sodium concentration in heparinized plasma.

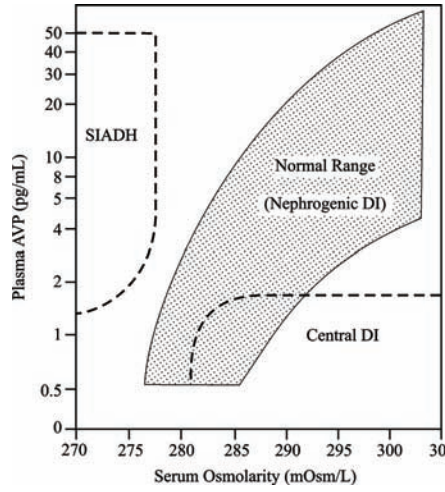

Figure 2. Quest Diagnostics Nichols Institute values for plasma AVP vs serum osmolarity in various clinical disorders. Values in nephrogenic diabetes insipidus (DI) fall within the normal range. Levels in central (pituitary) DI are low

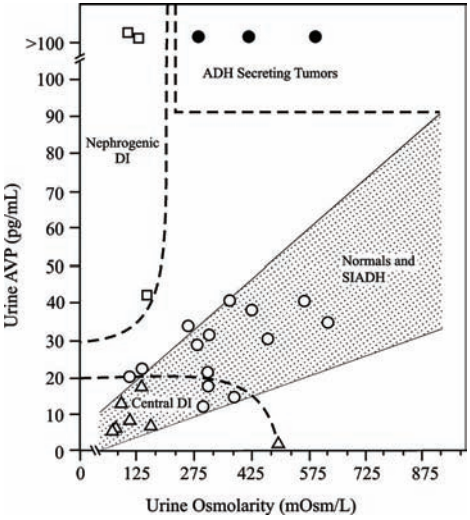

Figure 3. Quest Diagnostics Nichols Institute values for urine AVP and osmolarity. Results in patients with the syndrome of inappropriate antidiuretic hormone (SIADH) secretion overlap the normal range, except for selected patients with ADH-secreting tumors.

The differential diagnosis of true hyponatremia can be subdivided into those conditions occurring in patients with clinical evidence of increased, normal, and decreased extracellular fluid volume (Table 2). A plasma vasopressin measurement is not useful in distinguishing among these 3 sets of conditions.

Table 3. Differential Diagnosis of Hyponatremia

ECF Volume Status	Major Conditions	Relevant Laboratory Tests
Hypervolemic	Congestic heart failure	Liver function tests, creatinine, urine protein, BNP
	Cirrhosis and ascites	
	Nephrotic syndrome	
	SIADH	
Euvolemic	Hypothyroidism*	TSH, free T4
Hypovolemic	Dehydration with hypotonic fluid replacement	BUN, urine sodium
	Diuretic therapy, diarrhea, bulimia	BUN, urine sodium
	Adrenal insufficiency	Cortisol, basal & ACTH-stimulated
	Sodium wasting renal disorders (eg, medullary cystic disease)	Creatinine, urine sodium

SIADH, syndrome of inappropriate antidiuretic hormone (AVP); BNP, B-type natriuretic peptide.
*SIADH may also play a role in myxedematous hyponatremia.

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The syndrome of inappropriate vasopressin secretion (SIADH) also is a common cause of hyponatremia. In hyponatremic patients who appear euvolemic or mildly hypervolemic, SIADH must be considered. Classical criteria for SIADH include 1) hyponatremia and serum hypo-osmolality, 2) an inappropriately concentrated urine, and 3) normal effective intravascular fluid volume. Additionally, adrenal insufficiency, hypothyroidism, and renal disease should be excluded. The measurement of plasma or urine AVP can be used to confirm the diagnosis. The plasma AVP measurement is only informative when the sample is obtained when plasma osmolality is <270 mOsm/kg and serum sodium is <130 mEq/L. Certain caveats are important to keep in mind whenever measuring the plasma vasopressin concentration (Table 4).

Disorders of Potassium

The cause of hypokalemia is often clinically evident (eg, diuretic therapy or gastrointestinal loss), and no further specialized laboratory testing is required. However, in hypertensive, hypokalemic patients with suspected hyperaldosteronism, serum and urine measurements of aldosterone and plasma renin determinations (plasma renin activity or direct renin) are indicated, and dynamic testing may be required (see "Disorders of Adrenal Function" section)

The most common cause of hyperkalemia in clinical practice is renal insufficiency, especially when combined with certain drug effects (eg, potassium-sparing diuretics, beta-adrenergic blocking agents, angiotensin-converting enzyme inhibitors). Factitious causes of elevated serum potassium include hemolysis or thrombocytosis, which can be differentiated by proper sample handling and, when necessary, measurement of plasma potassium concentration. When primary adrenal insufficiency, isolated mineralocorticoid insufficiency, or hyporeninemic hypoaldosteronism must be considered, adrenal steroid hormone assays are essential (see "Disorders of Adrenal Function" section), and adrenal cortical stimulation testing is often indicated (see "Dynamic Test Application and Interpretation" section)

Table 4. Considerations in Plasma Vasopressin Measurement

Clinical
Hypotension and nausea increase release.
Nicotine and caffeine use increase release.
Many drugs alter vasopressin dynamics.
Analytical
Plasma samples must be obtained, processed, and handled with meticulous attention to optimal conditions.
Normal ranges are specific to each laboratory.
Values must be interpreted in light of simultaneous plasma and/or urine osmolality.

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PHEOCHROMOCYTOMA, MEDULLARY THYROID CARCINOMA, AND MULTIPLE ENDOCRINE NEOPLASIA

Pheochromocytoma

Pheochromocytoma occurs at any age independently and sporadically or as a feature of familial multiple endocrine neoplasia (MEN) syndromes, von Hippel-Lindau syndrome (VHL), neurofibromatosis-1 (NF-1), or familial pheochromocytomal paraganglioma. In children, catecholamine hypersecretion is also associated with neuroblastoma or ganglioneuromata due to mutations in the succinate dehydrogenase (SDH) gene family. Sixty percent to 70% of pheochromocytomas are sporadic and 20% to 30% are familial. Ninety percent of sporadic cases occur in the adrenal gland, and 90% of these are unilateral. Over 90% of extra-adrenal tumors occur in the abdomen (organ of Zuckerkandl, sympathetic ganglia, urinary bladder), and 10% occur elsewhere (chest, heart, carotid body, vagus nerves). Sporadic pheochromocytomas are usually solitary, well circumscribed, encapsulated, and slow growing. Familial pheochromocytomas are more likely to be multiple and bilateral (50%). Pheochromocytomas usually range in size from 3 to 5 cm, but tumors as large as 10 to 20 cm have been reported.

The clinical manifestations of pheochromocytoma vary in type and severity. The most common symptoms and signs include headache, hypertension, postural hypotension, tachycardia, palpitations, and sweating. Some patients have asymptomatic hypertension; many have intermittent symptoms; and severe cases may manifest weight loss, fasting hyperglycemia, weakness, fatigue, or seizures. Familial cases may present with a thyroid mass (medullary carcinoma).

Three types of MEN have been described: MEN 1, MEN 2A, and MEN 2B. These syndromes, including the hormones/neuropeptides that can be hypersecreted in each, are summarized in Table 1. MEN syndromes can involve the pituitary, parathyroid, thyroid, adrenal medulla, and pancreatic-gastrointestinal tissues. Pheochromoctyomas caused by familial MEN 2A and MEN 2B are transmitted as autosomal dominant traits and associated with gain of function mutations of the *RET* proto-oncogene on chromosome 10. The penetrance of medullary thyroid carcinoma (MTC) in these 2 types is 100% (See "Medullary Thyroid Carcinoma" below). Fifty percent of affected individuals also develop pheochromocytoma, and 20% develop hyperparathyroidism. Patients with MEN 2B present with associated mucosal neuromata and a marfanoid habitus as well.

Pheochromocytomas in MEN 2 patients, in contrast to sporadic forms, are rarely extra-adrenal or malignant and are diagnosed at a younger age. This has led to the practice of cortex-sparing adrenalectomy to avoid the risk of adrenal insufficiency. The tumors are bilateral in 50% of patients. The risk of recurrent pheochromocytoma in the contralateral gland in patients with unilateral disease is 20% to 30%. Patients with unilateral disease can now be managed via laparoscopic unilateral complete adrenalectomy. For bilateral disease, a unilateral cortical sparing procedure with removal of the entire contralateral gland is recommended.

Table 1. Hormones in Multiple Endocrine Neoplasia Syndromes

MEN Type	Parathyroid	Pituitary	Pancreas Gastrointestinal	Adrenal Medulla	Thyroid
MEN 1 (Werner Syndrome)	PTH	PRL GH ACTH	Gastrin Insulin Glucagon GHRH VIP SRIF PP		
MEN 2 or 2A (Sipple syndrome)	PTH			CAT Met ChrA	CT CEA ChrA *
MEN 2B†	PTH (rare)			CAT Met VMA ChrA	CT CEA ChrA *

ACTH, adrenocorticotropic hormone; CAT, catecholamines; ChrA, chromogranin A; CT, calcitonin; CEA, carcinoembryonic antigen; GH, growth hormone; GHRH, growth hormone releasing hormone; Met, metanephrines; PP, pancreatic polypeptide; PRL, prolactin; PTH, parathyroid hormone; SRIF, somatostatin; VIP, vasoactive intestinal polypeptide; VMA, vanillylmandelic acid.

*Ectopic hormones sometimes secreted by medullary thyroid carcinomas: serotonin, VIP, SRIF, ACTH.

†MEN 2B includes mucosal ganglioneuromas, marfanoid habitus.

Familial pheochromocytomas occur in 10% to 15% of patients with VHL, which is caused by autosomal dominant mutations in the *VHL* gene. VHL involves retinal, cerebellar, and spinal hemangioblastoma; renal cell carcinoma; pheochromocytoma; and pancreatic tumors. Familial pheochromocytomas also occur in 10% to 12% of patients with NF-1. Four percent to 12% of sporadic pheochromocytomas and up to 50% of familial pheochromocytomas are associated with mutated genes in the *SDHB* and *SDHD* genes. The *SDHD* gene is imprinted, and disease is only expressed when the mutation is inherited from the father.

Diagnosis of Pheochromocytoma

The diagnosis of pheochromocytoma is accomplished by documenting excessive secretion of catecholamines. Measurements of urinary total metanephrines and urinary catecholamines have the highest sensitivity. Plasma catecholamines also are sensitive markers, especially during an attack, but samples must be properly collected and processed for accurate results. Urinary VMA measurements have lower sensitivity. If levels of these several analytes exceed the values in Table 2, the diagnosis of pheochromocytoma is clear. Patients with SDHB-related paragangliomas often present with symptoms of the tumor mass rather than catecholamine excess, and the biochemical phenotype is hypersecretion of norepinephrine and/or dopamine (Ref. 14). The sensitivities of screening tests for pheochromocytomas are summarized in Table 3.

Table 2. Laboratory Criteria for Diagnosis of Pheochromocytoma

Test	Threshold
Plasma catecholamines (supine, resting)	≥2,000 pg/mL
Urine total metanephrines	≥1.8 mg/24-h
Urine vanillylmandelic acid	≥11 mg/24-h
Urine normetanephrine	≥156 µg/24-h

Bravo EL. *Endocr Rev.* 1994;15:356-368 (Ref. 4).

A variety of medications may produce false-positive results in measurements of urinary metanephrines and catecholamines. These include methyldopa (Aldomet®'), levodopa (Sinemet®', Larodopa®', Atamet®'), labetalol (Trandate®', Normodyne®'), sotalol (Betapace®'), tricyclic antidepressants, benzodiazepines, drugs containing catecholamines, amphetamines, withdrawal from clonidine (Catapres®'), and ethanol. Metyrosine (Demser®') or methylglucamine may produce false-negative results.

Genetic Testing for Pheochromocytoma

Twenty to 30% of pheochromocytomas are associated with germline mutations in one of several genes. These include the: 1) *RET* proto-oncogene, 2) von Hippel-Lindau (*VHL*) type 2 gene, 3) neurofibromatosis type 1 (NF1) gene, and 4) succinate dehydrogenase subunit genes (*SDHD*, *SDHB*, *SDHC*). In both paraganglioma and pheochromocytoma, far more germline mutations have been identified in the *SDHD* subunit than in the other succinate dehydrogenase genes; 97% of germline mutations in paraganglioma families are in the *SDHD* gene. *SDHB* mutations account for most of the remainder. *SDHC* mutation is a rare cause of pheochromocytoma.

Because of the high incidence of germline mutations in patients with pheochromocytoma, it is important to obtain a thorough clinical and family history, especially for patients younger than 50 years. Genetic counseling is important, and testing can be focused on a specific gene if the data suggest MEN

Table 3. Sensitivity of Screening Tests for Familial Pheochromocytoma

Test	Sensitivity (%)	Specificity (%)	Likelihood Ratio of Positive Test (95% CI)	Likelihood Ratio of Negative Test (95% CI)
Fractionated plasma metanephrines	97	85	6.3 (4.7-8.5)	0.04 (0.006-0.26)
24-Hour urinary total metanephrines and catecholamines (either test positive)	90	98	58.9 (22.1-156.9)	0.10 (0.03-0.29)

Sawka, et al. *J Clin Endocrinol Metab.* 2003;88:553-558 (Ref. 10).

2, VHL, or NF-1. When isolated pheochromocytoma involves the adrenal, mutations are most frequently in the *VHL* or *RET* genes. In extra-adrenal disease, the most frequent sites of mutations are the *SDHB* and *SDHD* genes. In the absence of germline mutations in the *VHL*, *SDHD*, *SDHB*, or *RET* genes, patients with pheochromocytoma, especially when bilateral, should be thoroughly checked for subtle manifestations of NF-1 (Ref. 15).

Medullary Thyroid Carcinoma (MTC)

The classification of MTC is summarized in Table 4. The MEN and familial MTC (FMTC) phenotypes are associated with gain of function mutations of the *RET* proto-oncogene, a tyrosine kinase receptor first identified and mapped to chromosome 10 through classical linkage analysis. Gain of function *RET* mutations causing MEN 2 were first identified in 1993. The most frequent mutations causing MEN 2A or FMTC occur in exons 10 and 11 of the *RET* gene. They convert 1 of 5 highly conserved cysteines at positions 609, 611, 618, 620, 634, 635, and 637 in the extracellular region of the receptor to another amino acid. Less frequent mutations in exons 13 to 15 of *RET* (which code for the tyrosine kinase domain of the protein) also cause FMTC and/or MEN 2A. Germline mutations of codon 918 cause ≥95% of MEN 2B cases. First-degree relatives (parents, siblings, and children) of affected individuals should be studied for the presence of this mutation because the clinical MEN 2B phenotype is not always obvious. Table 5 summarizes *RET* proto-oncogene mutations associated with MEN 2 and FMTC.

Long-term follow-up of hereditary MTC patients is essential to detect recurrence of disease in treated patients or the later appearance of pheochromocytoma or hyperparathyroidism. In MEN patients, pheochromocytoma is diagnosed after MTC in 90% of patients. Parathyroid hyperplasia is associated most commonly with codon 634 mutations and less frequently with codons 609, 611, 618, 620, 790, and 791 mutations. Parathyroid hyperplasia or adenoma in MEN 2A patients may be associated with symptoms of hypercalcemia or may be subclinical with only a mild increase in serum calcium levels. FMTC is defined as the presence of MTC in kindreds with 4 or more affected family members, and the onset of MTC commonly is delayed until 20 to 40 years of age. Thus, in many cases it is difficult to distinguish between MEN 2A and FMTC, because the FMTC kindred may have few individuals, incomplete histories, or a predominance of young individuals who have not reached the age for full penetrance of pheochromocytoma or hyperparathyroidism.

Table 4. Classification of Hereditary Medullary Thyroid Cancer

	Phenotype			
	MTC	MN	PCT	HPT
MEN 2A	100%	100%	50%	20%
MEN 2B	100%	N	50%	N
FMTC	100%	N	N	N

MTC, medullary thyroid carcinoma; MN, mucosal neuromata; PCT, pheochromocytoma; HPT, hyperparathyroidism; MEN, multiple endocrine neoplasia; FMTC, familial MTC; N, not present. Modified from Kouvaraki, et al. *Thyroid.* 2005;15:531 (Ref. 20).

Table 5. *RET* Gene Mutations and Associated Endocrine Disorders

Clinical Syndrome	Exon	Codon
MEN 2A/FMTC	10	609, 611, 618, 620
	11	634, 635, 637
	13	790, 791
	14	804
	15	891
FMTC	8	532, 533
	11	630
	13	768, 791
	14	804, 844
	16	912
MEN 2B	14	804 + 806, 804 + 904
	15	883
	16	918, 922

Kouvaraki, et al. *Thyroid.* 2005;15:531 (Ref. 20).

Hereditary MTC Diagnosis and Management

Prior to 1987, the only available test to detect MTC in at-risk patients was measurement of serum calcitonin with or without stimulation. Since then, DNA diagnosis has become the procedure of choice. Identification of a mutation in the *RET* protooncogene indicates that the affected individual has a >90% probability of developing MTC. The optimal treatment strategy is to prevent MTC in children with *RET* gene mutations by performing early thyroidectomy before malignant transformation occurs.

There is now a large experience correlating *RET* mutations with MTC aggressiveness, and high risk mutations have been identified. These are ranked as level 1 to 3 with 3 being highest risk (Table 6). For the highest risk group (mutated exons 883 or 918 in MEN 2B patients), thyroidectomy is recommended before age 6 months or preferably within the first month of life. Thyroidectomy is recommended before 5 years of age for level 2 risk patients and at 5 to 10 years of age for level 1 risk patients. An alternative approach in lower risk patients is to initiate calcitonin-calcium stimulation testing of gene mutation carriers at age 4 or 5 years with removal of the thyroid gland at the time of a positive test. Pentagastrin also has been used for calcitonin stimulation testing but is no longer available in the United States. Calcium stimulation testing has been shown to be equally effective (Ref. 21).

Table 6. Recommended Management for Patients with MTC

	Level 1 High Risk	Level 2 High Risk	Level 3 Highest Risk
Mutated codons	609, 768, 790, 791, 804, 891	611, 618, 620, 634	883, 918
Phenotype	MEN 2A, FMTC	MEN 2A, FMTC	MEN 2B
Recommended Management	Thyroidectomy 5-10 y of age	Thyroidectomy <5 y of age	Thyroidectomy 1-6 mo of age

Kouvaraki, et al. *Thyroid.* 2005;15:531 (Ref. 20).

Test Application and Interpretation

Genetic testing is not infallible, as laboratory or sampling errors can occur. If genetic testing is used as the sole determinant of gene carrier status and hereditary MTC, the genetic test should be repeated on a separate peripheral blood sample. It is reasonable to exclude an individual with 2 or more negative genetic test results from further screening. It is important to remember that *RET* proto-oncogene mutations also predispose to adrenal medullary and parathyroid disease, manifestations that generally develop later. Additional testing should be performed for these conditions.

In kindred with hereditary MCT but no identifiable *RET* mutations, there is no alternative to stimulation testing. Traditionally, this was done with calcium-pentagastrin stimulation; however, pentagastrin is no longer commercially available. MTC is associated with hypersecretion of calcitonin, manifest by either increased circulating concentration and/or increased calcitonin response to calcium stimulation. The stimulation test usually is conducted in the morning with the patient supine and NPO after midnight; 2 mg of elemental calcium per kg body weight is infused intravenously over 1 minute. Blood samples for calcitonin measurements are collected at 0, 2, 5, and 10 minutes. Normal values for adults (21-50 years) using the Nichols Institute Diagnostics immunochemiluminometric assay are shown in Table 7.

Sporadic MTC Diagnosis and Management

MTC represents 4% to 10% of all malignant thyroid neoplasia, and 75% are sporadic rather than familial. The prognosis generally is poor, because lymph node involvement occurs early even when the tumor is small. Final diagnosis is based on fine needle aspiration biopsy or surgical histology, but elevated serum calcitonin concentrations serve as an important biomarker. Costante, et al, in a recent study of 5817 patients (1111 males, 4706 females) consecutively diagnosed with thyroid nodules, identified 282 with MTC (Ref. 21). Basal and pentagastrin-stimulated serum calcitonin (CT), tumor histology assessed by fine needle aspiration biopsy, and surgical histology (C-cell hyperplasia or MTC) results in these patients are summarized in Table 8. Basal and stimulated CT levels >100 pg/mL were indication for surgery. Positive predictive values (PPV) for basal CT levels were 100% for values >100 pg/mL, 25% for values 50-100 pg/mL, and 8% for values 20-50 pg/mL. PPV for pentagastrin-stimulated CT test results >100 pg/mL was 40%.

It is not possible to determine a priori whether an individual with apparent sporadic MTC has hereditary or sporadic disease. Available information indicates that 6% to 7% of apparent sporadic MTC have germline mutations of

Table 7. Calcitonin-Calcium Stimulation Test*

	Men	Women
Basal	<8.0	<4.0
2 minutes	15-205	≤35
5 minutes	10-125	≤25
10 minutes	4-125	≤20

*Values in pg/mL, 2 SD range; ICMA method. Data from Nichols Institute Clinical Correlations Department.

Table 8. Basal and Pentagastrin-stimulated Calcitonin Results vs Histologic Diagnosis

Basal CT pg/mL	No. Patients	PG-CT % Positive	Histologic Diagnosis		
			FNAB MTC	Surgical Specimen	
				CCH	MTC
>100	9	ND	6	0	9
≥59-99	8	50	0	2	2
≥20-49	49	25	1	4	4
>10-19	216	0.5	0	1	0

CT, calcitonin; PG-CT, pentagastrin-stimulated calcitonin; FNAB, fine needle aspiration biopsy; MTC, medullary thyroid carcinoma; CCH, C-cell hyperplasia; ND, not determined. From Costante et al. *J Clin Endocrinol Metab.* 2007;92:450 (Ref. 21).

the *RET* proto-oncogene. It is important to identify these individuals so that screening can be provided to other family members. For this reason, it is now recommended by the American Society of Clinical Oncology and the National Comprehensive Cancer Network that all patients with apparently sporadic MTC be tested for germline *RET* gene mutations.

Approximately 25% of patients with apparent sporadic MTC have a somatic mutation in codon 918 of the *RET* proto-oncogene. At present, the finding of a somatic mutation has no specific clinical ramifications, although there are reports correlating the presence of this particular mutation with more aggressive disease. However, more information is required before a definitive statement can be made.

Most (>90%) of patients with apparent sporadic medullary carcinoma have no germline or somatic mutation of the *RET* proto-oncogene. It is likely that mutations in other genes will be found to be causative for the transformation in these patients.

MEN Type 1

MEN type 1 is caused by mutations in the gene encoding the tumor suppressor protein menin located on chromosome 11q13. Patients with MEN type 1 inherit a germline mutation in one allele of this gene and develop clinical disease when the second allele undergoes a (inactivating) somatic mutation (the 2-hit model for tumorigenesis). Although the exact genetic function of menin is not totally elucidated, emerging data indicate it is involved with regulation of gene transcription, cell proliferation, apoptosis, and genome stability. The incidence of MEN type 1 syndrome has been estimated to be 0.25% (1 in 400 individuals) based on randomly chosen postmortem studies. About 80% of patients develop clinical manifestations by their fifth decade. The spectrum of tumor types is summarized in Table 9. Hyperparathyroidism is the most common feature of MEN type 1, occurring in some 95% of patients. The diagnostic approach to these patients is reviewed in the parathyroid gland section.

Test Application and Interpretation

Table 9. Endocrine Tumors Associated with MEN Type 1

Parathyroid	Pituitary
Hyperplasia	Prolactinoma
Pancreas	GH-secreting
Gastrinoma	ACTH-secreting
Insulinoma	Chromophobe
Glucagonoma	Other
VIPoma	Adrenocortical
PPoma	Carcinoid
	Lipomas

Pancreatic tumors occur in 30% to 80% of MEN type 1 patients. Gastrinomas occur in about half of the affected individuals. They are associated with increased gastric acid production, recurrent peptic ulceration, diarrhea, and steatorrhea (Zollinger-Ellison syndrome). When plasma gastrin levels are elevated (>200 pg/mL), the diagnosis is usually clear. In borderline cases, a secretin stimulation test is useful, and a rise in secretin level of 200 pg/mL over the basal level is considered a positive test with 90% sensitivity and specificity. CT or MRI imaging or selective angiography can be utilized to localize the tumor. Hyperthyroidism may also occur in these patients.

Insulinomas represent 30% to 40% of the pancreatic tumors in MEN type 1 patients. They are associated with gastrinoma in 10% of patients. The diagnosis may be difficult, as reviewed in the "Disorders of Carbohydrate Metabolism" section.

Glucagonomas, vasoactive intestinal peptide-secreting adenomas (VIPomas) and pancreatic polypeptide-secreting adenomas (PPomas), are rare. The majority of them is malignant and in many instances has metastasized by the time of diagnosis. Hormone measurements can serve as tumor markers after surgery.

Patients with glucagonoma may present with a skin rash (necrolytic migratory erythema), stomatitis, anemia, and weight loss or may have only hyperglycemia and hyperglucagonemia. Symptomatic patients' glucagon levels are usually above 500 pg/mL. Milder elevation of glucagon, while possible in symptomatic patients, may be either false-positive due to stress, hypoglycemia, acute pancreatitis, renal or hepatic failure, or due to Cushing's syndrome and carcinoids.

Patients with VIPomas characteristically manifest watery diarrhea, hypokalemia, and achlorhydria (the Werner-Morrison or WDHA syndrome). The diagnosis is confirmed by finding markedly increased levels of plasma VIP (>200 pg/mL). Hyperparathyroidism often is present.

Pancreatic polypeptide hypersecretion is observed in about half of patients with islet cell tumors. Pure PPomas are rare and are not associated with a well-defined clinical syndrome.

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Test Application and Interpretation

DYNAMIC TESTS

Every effort has been made to ensure accuracy in these recommendations, but clinicians must use their judgment and refer to specific pharmaceutical resources to determine appropriate drug dosages for their patients.

Adrenal Dynamic Tests

ACTH Stimulation Test, Standard[1-3]

Indication: Suspected adrenal insufficiency; suspected adrenal biosynthetic defect or ACTH unresponsiveness

Medication: 10 µg/kg, maximum 250 µg (0.25 mg), cosyntropin (Cortrosyn®', synthetic 1-24 ACTH) by IV bolus; dilute in 2 to 5 mL physiological saline. Intramuscular dosing is equally effective; dilute in 1 mL physiological saline.

Sampling: Serum cortisol at 0 and 30 or 60 minutes

Interpretation: The usual normal response is an approximate doubling of the basal level, provided that the basal level does not exceed the normal range. Analysis of 8 reported studies including 122 patients indicated sensitivities of 97% and 57% respectively at 95% specificity for diagnosis of primary and secondary adrenal insufficiency using a serum cortisol cutoff of 15 µg/dL.

Comments: Normal response excludes primary adrenal insufficiency. Patients with secondary (ACTH) adrenal insufficiency usually show a blunted response but may have a normal result. Disparate levels of baseline precursor and product steroids (precursor > product) localize enzyme dysfunction in severe CAH cases. The ratio of precursor to product tends to be similar at baseline and peak sampling times. In children with milder defects, localization is defined by substantially disproportionate fold increases in precursor and product steroid levels after ACTH.

ACTH Stimulation Test, Prolonged[1,2]

Indication: Suspected secondary adrenal insufficiency; differentiates primary from secondary adrenal insufficiency

Medication: 0.5 IU/m^2 corticotropin (Acthar®' gel) intramuscularly every 12 hours for 3 days or 10 µg/kg cosyntropin (Cortrosyn) intravenously in 100-250 mL of physiological saline over 8-12 hours for 1 to 2 days; maximum, 250 µg.

Sampling: Serum cortisol before and 2 hours after each injection or infusion for 3 days and/or 24-hour urine samples for 4 days (baseline + 3 stimulation days)

Interpretation: Serum cortisol to exceed 25 µg/dL. Urine 17-hydroxy-corticosteroids or free cortisol values usually increase 2-fold.

Comments: May give dexamethasone (20 µg/kg/d) to prevent adrenal crisis – will not interfere with test. Normal subjects and patients with secondary adrenal insufficiency will respond to prolonged stimulation.

ACTH Stimulation Test, Low Dose[1,3]

Indication: Suspected adrenal insufficiency, biosynthetic enzyme defect, or ACTH unresponsiveness

Medication: 1.0 µg/1.73 m^2 cosyntropin (Cortrosyn, 1-24 synthetic ACTH) by IV bolus in 1.0 mL saline

Sampling: Blood for measurements of cortisol at 0, 30, and 40 minutes

Interpretation: Serum cortisol should be ≥15 µg/dL.

Comments: The low dose test is useful in infants, avoiding under diagnosis of hypothalamic-pituitary-adrenal axis impairment. It is particularly useful in assessing adrenal responsiveness before discontinuation of dexamethasone treatment. Glucocorticoid should be withheld for 12 hours prior to testing. In older patients, the low dose test has equal sensitivity to the standard ACTH test.

Aldosterone Suppression Test[1,2,4]

Indication: Suspected primary hyperaldosteronism

Medication: IV saline loading (2 L of 0.9% NaCl infused over 4 hours) or oral sodium loading for 3 to 4 days (urine sodium excretion >200 mEq/24-h) or administration of 0.1 mg fludrocortisone acetate q 6 hours plus NaCl x 4 days

Sampling: Aldosterone and direct renin or plasma renin activity (PRA) are measured before and after suppression.

Interpretation: In normal subjects, the serum aldosterone is <5 ng/dL after suppression. PRA is suppressed to the lower limit of detection.

Comments: None

CRH Stimulation Test[1,2]

Indication: Suspected pituitary ACTH deficiency; suspected pituitary Cushing's; rule out ectopic disease

Medication: 1.0 µg/kg (100 µg/1.73 m^2; maximum dose 100 µg) synthetic ovine or human CRH administered as IV bolus over 30 seconds. Patient fasting 4 hours.

Sampling: Blood measurements of cortisol and ACTH at -5 and -1 minutes before CRH and at 15, 30, 45, and 60 minutes after CRH.

Interpretation: Baseline ACTH increases 20% to 40% in 95% of normal subjects. Peak values (usually in the 20 to 100 pg/mL range) are observed at 30 to 60 minutes. Serum cortisol peaks at 30 to 60 minutes in the 20 to 25 µg/dL range.

Comments: The test can be conducted in the AM or PM, but in normal subjects the responses vary with time of day. The ACTH increment is similar morning and evening, but peak values vary with baseline (higher in AM). Cortisol levels peak at similar values morning and evening. Patients with pituitary ACTH deficiency have subnormal responses; those with hypothalamic disease tend to have augmented and prolonged ACTH responses with reduced cortisol response. Patients with primary adrenal insufficiency have high baseline ACTH, an augmented response to CRH, and low cortisol levels before and after CRH. Patients with Cushing's disease (pituitary Cushing's) usually show a >20% rise in cortisol and a >50% increase in ACTH. Patients with adrenal causes of Cushing's syndrome or ectopic ACTH syndrome do not respond. Rarely, bronchial carcinoids producing ACTH may respond to CRH.

CRH Stimulation Test, Adrenal Venous Sampling[5]

Indication: Distinguish between an adenoma or unilateral and bilateral hyperplasia in patients with primary aldosteronism. The test also is helpful when imaging studies fail to unequivocally identify an adenoma or when the identified adenoma is <1 cm in size.

Medication: 50 µg per hour of cosyntropin (Cortrosyn) is infused intravenously 30 minutes prior and continuously throughout the procedure.

Sampling: In this standard protocol, measurements for both cortisol (µg/dL) and aldosterone (ng/dL) are performed in serum samples obtained from the inferior vena cava (IVC) and each of the adrenal veins.

Interpretation: A catheterization is considered successful if the adrenal vein cortisol level is 5-fold higher than the level in IVC. A lateralizing response is defined as a serum aldosterone/cortisol ratio 4-fold higher in the adrenal vein draining the secreting side. A ratio of <3 is consistent with bilateral aldosterone hypersecretion, while a ratio between 3 and 4 is an area of overlap.

Comments: Determination of aldosterone/cortisol ratio in samples of adrenal venous blood, obtained by an experienced radiologist, is the "gold standard" test. Unilateral disease is associated with a marked increase in the aldosterone/cortisol ratio on the side of the tumor, whereas there is little difference between the 2 sides in patients with bilateral hyperplasia. In a study of 163 men and 40 women with hyperaldosteronism, 56.7% of patients had a unilateral source of aldosterone secretion. The cutoff described above was able to lateralize the secreting site with 95% sensitivity and 100% specificity.

CRH Stimulation Test, Petrosal Venous Sinus Sampling[1,6]

Indication: Differentiation between ectopic and pituitary causes of Cushing's disease. This procedure can better distinguish between ectopic and pituitary ACTH hypersecretion than dexamethasone suppression, but it is invasive, may be associated with serious complications, and therefore should be performed by an experienced radiologist. This procedure appears to be less useful for lateralization of the tumor in cases in which the ACTH secretion appears to be of pituitary origin.

Medication: ovine CRH (1 µg/kg body weight) administered as IV bolus

Sampling: Catheters are inserted via the jugular or femoral veins into both inferior petrosal veins. Blood is obtained from a peripheral vein via a third catheter or from a port in the iliac vein. Blood samples are drawn simultaneously from both inferior petrosal sinuses and the peripheral vein for plasma ACTH. At least 4 sets of 3 samples each are obtained: 2 sets are drawn immediately before injection of CRH as a baseline and 2 more are drawn between 2 and 3 and between 5 and 6 minutes after peripheral intravenous injection of CRH.

Interpretation: A central-to-peripheral plasma ACTH gradient of 2.0 before CRH administration, or 3.0 after CRH, is diagnostic of a pituitary source of ACTH. A gradient of 1.4 to 1.5 between the 2 sinuses may predict the lateral site of the adenoma.

Comments: In 14 studies, 759 of a total of 800 patients with proven Cushing's disease were correctly identified (95% sensitivity), while 115 of 124 with presumed or proven ectopic ACTH secretion were correctly identified (93% specificity). False negatives may occur in episodic Cushing's disease or in aberrant drainage through an abnormal petrosal sinus on the side of the adenoma. The lateralization of the adenoma in pituitary Cushing's through this procedure is of dubious value since it is only 70% accurate. The procedure is not indicated in subjects with a pituitary adenoma >6 mm and a response suggestive of pituitary Cushing's on the CRH or dexamethasone tests.

Dexamethasone Suppression Tests[6]

Indication: Suspected endogenous Cushing's syndrome or dexamethasone-suppressible hyperaldosteronism (DSH). Differentiate Cushing's disease (pituitary) from ectopic source. Suggest a major adrenal source of androgen in polycystic ovarian disease.

Overnight Suppression Test

Medication: 20 µg/kg (1 mg in adults) dexamethasone at 11-12 PM

Sampling: Serum cortisol at 8 AM

Interpretation: Suppression of 8 AM serum cortisol to <2 µg/dL effectively excludes Cushing's syndrome. A cortisol level >2 µg/dL merits further evaluation.

2-Day, Low Dose Suppression Test

This test has been shown to have a sensitivity of 79%, a specificity of 74%, and a diagnostic accuracy of only 71% in patients with mild Cushing's syndrome. The test is complex and difficult to administer and is no longer recommended.

Carbohydrate Metabolic Dynamic Tests

Glucose Tolerance Test[7]

Indication: Suspected impairment of glucose tolerance

Test Application and Interpretation

Medication: High carbohydrate diet (50% of calories) for 3 days before test. Patient NPO after midnight; glucose solution 1.75 g/kg (maximum dose 75 g) orally.

Sampling: Blood glucose and insulin measurements at 0, 60, 120, and 180 minutes. Free insulin or C-peptide can be measured in subjects with insulin antibodies.

Interpretation:

Table 1. Normal Adult Levels*

Time (min)	Glucose (mg/dL)	Insulin (μU/mL)	C-Peptide (ng/mL)
0	65-109	<17	0.8-3.1
30	64-178	6-86	2.1-10.8
60	53-153	8-112	2.3-11.9
90	53-134	5-68	1.7-11.5
120	51-113	5-55	1.2-8.6
150	42-108	3-46	1.0-8.5
180	60-98	3-20	1.0-5.3
240	62-106	<15	0.7-4.3
300	71-106	<8	0.7-3.2

*Data from Quest Diagnostics Nichols Institute Clinical Correlations Department.

Table 2. Diagnostic Criteria

Diagnosis	Glucose (mg/dL)	Time (min)
Diabetes mellitus	≥126	0
	≥200	60, 120, 180
Impaired glucose tolerance	100-125	0
	140-199	120
Gestational diabetes	>105	0
	>165	120

Comments: The test should be postponed for 1-2 weeks after any illness. Water can be taken freely during the test; ambulation is permitted. Glucose responses between normal and diabetic diagnostic levels suggest impaired glucose tolerance. Children with overt type 1 diabetes have a blunted or absent insulin response. See text for more detail.

Pancreatic Hormone Response Test[8]

Indication: Suspected abnormality in pancreatic islet cell function

Medication: High carbohydrate diet for 3 days before test. Patient NPO after midnight. Oral administration of 1.75 g/kg (maximum dose 75 g) glucose solution

Test Application and Interpretation

Sampling: As shown in Table 3.

Interpretation:

Table 3. Normal Adult Concentrations (95% Range)

Time minutes	Glucose[a] mg/dL	Insulin[a] μU/mL	Proinsulin[b] pmol/L	C-Peptide[a] ng/mL
0	65-109	<17[c]	≤18.8	0.8-3.1[d]
30	64-178	6-86	≤32.9	2.1-10.8
60	53-153	8-112	3.9-44.6	2.3-11.9
90	53-134	5-68	≤44.5	1.7-11.5
120	51-113	5-55	2.9-33.2	1.2-8.6
150	42-108	3-46	≤31.8	1.0-8.5
180[e]	60-98	3-20	≤14.6	1.0-5.3
240[e]	62-106	<15	≤12.3	0.7-4.3
300[e]	71-106	<8	≤13.4	0.7-3.2

Data from Quest Diagnostics Nichols Institute Clinical Correlations Department. Apparently healthy Quest Diagnostics employees, ambulatory, community dwelling, non-medicated, fasting overnight. Exclusion criteria include abnormal fasting glucose, AST, ALT, BUN, creatinine, BMI>30 kg/m^2. M, males; F, females.

[a]N=43: 26 M, 17 F; 18-50 years of age.

[b]N=41: 23 M, 18 F; 20-51 years of age.

[c]N=131: 60 M, 71 F; 18-55 years of age.

[d]N=109: 54 M; 55 F; 18-55 years of age.

[e]N=18: 10 M, 8 F; 20-51 years of age; all 4 analytes.

Comments: Methods used for development of normal ranges are as follows: insulin, Immulite® ICMA; proinsulin, EIA IBL; C-Peptide, Immulite ICMA.

Prolonged Fasting Test[9,10]

Indication: Suspected fasting hypoglycemia, insulin hypersecretion, or counter-regulatory hormone deficiency

Medication: None. Water, diet soft drinks, and unsweetened tea are allowed. Ambulation is permitted. Fasting for 72 hours may be necessary.

Sampling: Blood measurements of glucose, insulin, and C-peptide every 4-6 hours and hourly as the blood glucose level falls below 50 mg/dL. Samples should be drawn and the test terminated whenever symptoms appear. To assess counter-regulatory hormones, blood for measurements of cortisol, growth hormone, IGF-I, glucagon, and/or catecholamines can be collected at baseline and at the time of hypoglycemia. Tests for lactate, ketones, and free fatty acids are helpful for the differential diagnosis of hypoglycemia in children.[7]

Interpretation: Blood glucose concentrations should remain above 45 mg/dL. Insulin values are low (<10 μU/mL) with blood glucose levels <60 mg/dL. The normal ratio of fasting insulin (μU/mL) to plasma glucose (mg/dL) is 0.3 or less.

C-peptide levels should fall to ≤0.6 ng/mL. Levels of counter-regulatory hormones increase in the presence of hypoglycemia.

Comments: The patient should be hospitalized or observed constantly in an outpatient environment for the test. There is a continuing risk of hypoglycemia. IV access should be maintained, and glucose for IV infusion must be available.

Gonadal Dynamic Test

GnRH Stimulation Test[11,12]

Indication: Suspected central precocious puberty (CPP); suspected pituitary gonadotropin deficiency; monitor long acting GnRH analogue therapy

Intravenous GnRH

Medication: 2.5 µg/kg, up to 100 µg maximum, rapid intravenous injection of GnRH (Factrel®, Wyeth® Ayerst, available in 0.1 or 0.5 mg vials)

Sampling: Blood samples for measurements of serum LH and FSH concentrations are obtained immediately before and at 30, 45, and 60 minutes after GnRH injection. A single 30-minute LH sample can be used to monitor GnRH analogue therapy.

Interpretation: Prepubertally, levels increase 2- to 4-fold with a peak LH/FSH ratio approximating 0.7. Postpubertally, LH levels increase 6- to 10-fold and FSH 4- to 6-fold with a mean peak LH/FSH ratio approximating 3.5. Cavallo et al describe >90% sensitivity and >80% specificity for a single LH concentration cutoff of 15 IU/L for the diagnosis of central precocious puberty (CPP).[11]

Subcutaneous GnRH

Medication: 100 µg GnRH (Factrel) administered subcutaneously

Sampling: Draw blood for serum LH and FSH measurements at -15 and 0 minutes and at 40 minutes after GnRH.

Interpretation: Eckert et al report the efficacy of the single sample subcutaneous test for the diagnosis of CPP.[12] They observed that LH and FSH values generally remain below 5 IU/L in normal prepubertal children; the FSH response usually exceeds the LH response. With the onset of puberty, there is a brisk LH response; values increase 4- to 6-fold above baseline and usually exceed 10 IU/L. An ICMA LH value ≥10 IU/L after GnRH is strong evidence for CPP in a prepubertal age female. Levels in the 5-10 IU/L range are suggestive of CPP.

Comments: The LH response probably is most useful for differential diagnosis. The test does not usually differentiate hypothalamic and pituitary disorders and does not reliably differentiate gonadotropin deficiency from constitutional delay in children with delayed puberty.

Test Application and Interpretation

Growth Hormone Dynamic Tests

Glucose Suppression Test[1-13]

Indication: Suspected autonomous growth hormone (GH) hypersecretion

Medication: 1.75 g/kg body weight (maximum 75 g) oral glucose after overnight fasting (or NPO after midnight)

Sampling: Serum glucose and GH measurements at 0, 30, 60, 90, and 120 minutes

Interpretation: GH levels normally suppress to <1 ng/mL. Failure to suppress in the presence of normally increased blood glucose suggests a pituitary adenoma.

Comments: Water may be given during the test, and ambulation is allowed. An adequate diet should be provided for 3 days prior to testing. Failure to suppress is observed in most patients with acromegaly. However, severe liver disease, chronic renal disease, uncontrolled diabetes mellitus, malnutrition, Laron syndrome, thyrotoxicosis, or L-dopa ingestion may be associated with inadequate GH suppression.

Growth Hormone Stimulation Test[14-18]

Indication: Suspected growth hormone (GH) deficiency

Medication: Single agents include

- Arginine, 0.5 g/kg (30 g maximum) L-arginine hydrochloride 10% solution IV over 30 minutes
- ITT: Insulin, 0.10 U/kg IV (0.05 U/kg if high degree of suspicion) to achieve a target blood sugar level of <40 mg/dL
- Levodopa, 125 mg orally if <15 kg (250 mg if <30 kg or 500 mg if >30 kg)
- Propranalol, 0.75 mg/kg (40 mg maximum) orally
- Clonidine, 5 µg/kg (300 µg maximum) orally
- GHRH, 1.0 µg/kg IV bolus
- GHRH+arginine: GHRH bolus first followed by arginine infusion.

All medications administered after overnight fast.

Sampling: Blood GH measurements at 0, 30, 60, 90, and 120 minutes

Interpretation: Insulin tolerance test (ITT) and GHRH-arginine test are the preferred tests for diagnosing GH deficiency in adults. When peak GH cutpoints of 5.1 ng/mL for the ITT and 4.1 ng/mL for the GHRH-arginine tests are employed, these tests show both high sensitivity (96% and 95%, respectively) and specificity (92% and 91%, respectively) in diagnosing GH deficiency. In children, the ITT remains the "gold standard" with an accuracy for predicting GH deficiency ranging from 85% at a GH cutoff of 10 ng/mL to 100% at a cutoff

of 3 ng/mL. However, it is seldom utilized, and the GHRH/arginine protocol is preferred. In recent years, most pediatric endocrinologists in North America have adopted a GH cutoff of 10 ng/mL for diagnosing GH deficiency.

Comments: In adults, a rigorous comparison of test results using ITT, GHRH+arginine, arginine, L-dopa, and arginine/ L-dopa protocols in the same patients revealed poor accuracy of the latter 3 tests. In children, there are no such studies comparing efficacy of the most frequently utilized single agents to the ITT or GHRH+arginine tests, and the sensitivity (positive predictive value) of the commonly used GH stimulation tests vary from about 40% using a GH cutoff of 10 ng/mL to 80% with a cut off of 2.5 ng/mL. In both children and adults, the GHRH component may cause false-positive GHRH+arginine test results in patients with GH deficiency of hypothalamic origin, since the pituitary is fairly intact and may be responsive. In such an instance, either the ITT or arginine alone can be utilized.

Multiple Endocrine Neoplasia Dynamic Test

Secretin Stimulation Test[19]

Indication: Suspected gastrinoma (Zollinger-Ellison syndrome)

Medication: 2 U/kg secretin administered intravenously as a bolus injection. Patients NPO after midnight. Patients off antacids and anticholinergic medications for 12 hours prior to test. Proton pump inhibitors should not be discontinued owing to possible complications (eg, acute bleeding and/or perforation).

Sampling: Gastrin measurement at baseline, 2, 5, 10, and 20 minutes

Interpretation: Minimal changes (<30%) in serum gastrin occur in normal subjects or those with common duodenal ulcer.

Comments: Patients with gastrinoma show increases in serum gastrin of at least 200 pg/mL or 50% by 5 or 10 minutes. The sensitivity is as high as 85% for gastrinoma.

Posterior Pituitary Dynamic Tests

Combined Anterior Pituitary (CAP) Test[20]

Indication: Assessment of pituitary reserve

Medication: CRH, 1.5 µg/kg (100 µg/1.73 m^2); GHRH, 1.5 µg/kg (100 µg/1.73 m^2); GnRH, 2.5 µg/kg (100 µg maximum); TRH, 7 µg/kg (500 µg maximum), IV, sequentially over 30 seconds using separate syringes

Sampling: Blood measurements of ACTH, GH, LH, FSH, TSH, and prolactin at

baseline, 15, 30, 60, 90 and 120 minutes

Interpretation: Responses vary with age, time of day, and target gland hormone levels. See "Alphabetical Test" section.

Comments: The CAP is usually used as a preliminary test. It may be useful to assess pituitary function after pituitary surgery, pituitary or cranial radiation, or head trauma, or to assess patients on chronic hormone replacement therapy (after hormone withdrawal). Various combinations of factors can be employed for testing selected pituitary hormone reserves.

Water Deprivation Test[21]

Indication: To differentiate among the causes of polyuria (not attributable to hyperglycemia)

Procedure: After an overnight fast, urine and plasma samples for osmolality should be obtained, followed by discontinuance of fluid intake. Hourly weights should be obtained, urine volumes should be measured, and hourly urine and plasma samples should be obtained for osmolality determinations. Central may be differentiated from nephrogenic diabetes insipidus by subsequent administration of 1 μg dDAVP (desmopressin) intravenously or 10 μg intranasally.

Interpretation: If the urine osmolality does not exceed 300 mOsm/kg before the plasma osmolality is >295 mOsm/kg or the serum sodium is >143 mmol/L, diabetes insipidus is diagnosed and primary polydipsia is excluded. Alternatively, the urine osmolality may be measured hourly, and once it has not risen by more than 30 mOsm/kg above the preceding hour, the plasma osmolality and serum sodium are measured. After subsequent administration of dDAVP, a rise in urine osmolality of >9% indicates normal renal responsiveness. The measurement of the plasma and urine vasopressin concentrations provides additional useful diagnostic information in cases with borderline urine and plasma osmolality responses.

Comments: Fluid restriction in patients with diabetes insipidus may cause severe extracellular fluid volume depletion. The test should be performed under careful supervision and terminated if the patient loses more than 3% of body weight. If urine volume has previously exceeded 100 mL/kg/d, overnight fasting is contraindicated, and the entire test should be initiated during the daytime.

Thyroid Dynamic Tests

Calcitonin-Calcium Stimulation Test[8]

Indication: Suspected medullary thyroid carcinoma

Medication: NPO after midnight. With the patient supine, give 20 mg/kg calcium gluconate (2 mg elemental calcium) intravenously over 1 minute.

Sampling: Blood calcitonin measurements at 0, 2, 5, and 10 minutes

Interpretation:

Table 4. Calcitonin Response in Normal Young Adults

	Calcitonin (pg/mL)	
	Males	Females
Basal	<8	<4
2 minutes	15-205	≤35
5 minutes	10-125	≤25
10 minutes	4-125	≤20

Comments: 2 mg elemental calcium also can be given as calcium lactate (13% elemental Ca) or calcium chloride (27% elemental calcium). Calcium infusion may produce generalized flushing, a feeling of warmth, the urge to urinate, and a sensation of gastric fullness lasting 1-5 minutes. Calcium should not be allowed to infiltrate subcutaneous tissues.

Thyrotropin Releasing Hormone (TRH) Stimulation Test[22,23]

Indication: To assess pituitary thyrotropin (TSH) and prolactin reserves; to establish TSH suppression by thyroid hormone; to confirm endogenous hyperthyroidism; to monitor thyroid hormone suppression therapy; to detect TSH, GH, LH, or FSH responses in patients with these secretory pituitary tumors.

Medication: With the patient recumbent, administer 200-500 µg (5-7 µg/kg/BW in children) TRH intravenously as a bolus.

Sampling: Obtain blood samples before and at appropriate time intervals after TRH administration. For assessment of pituitary TSH reserve, 30- and 60-minute samples are typically obtained; for prolactin, 15- and 30-minute samples. For assessment of TRH suppression, a single 30-minute sample is adequate.

Interpretation: Normally, the serum TSH concentration rises to the 7-30 mU/L range following TRH administration (7-35 mU/L in infants). A peak value between 0.1 and 7 mU/L is considered "blunted." The increase in TSH should be at least 6 mU/L in males and females under 40 years of age and 2 mU/L in males over 40 years. Prolactin levels increase 2- to 3-fold.

Comments: In addition to hypopituitarism and hyperthyroidism, blunted TSH responses may occur in nonthyroidal illness, depression, Cushing's disease, and acromegaly and with several medications. Transient nausea, sensation of warmth, flushing, metallic taste, and/or urge to micturate, typically lasting less than 1 minute, are frequently experienced by patients. Transient hypertension or hypotension may also occur. Measurement of the nocturnal TSH surge is also helpful in the diagnosis of hypothalamic hypothyroidism.

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International System of Units

SI Units

International System of Units (SI Units)

Système International d'Unités, or SI units, are an extension of the meter, kilogram, second, ampere (MKSA) system proposed in 1950. In 1966, the International Federation of Clinical Chemistry (IFCC) recommended the use of selected, or favored, SI units for the clinical laboratory: whenever possible, use *mole* and *liter* units to express concentration, and express whole-unit multiples of three as powers of ten (eg, millimole = 10^{-3} moles, micromole = 10^{-6} moles, nanomole = 10^{-9} moles, picomole = 10^{-12} moles). This approach was designed to provide clearer quantitative relationships among molecular species and some standardization of databases.

This partial application of SI units to laboratory data has been accepted by many scientific journals and is used increasingly in day-to-day clinical medicine. The operative words are "whenever possible." In many instances the molecular weight or the homogeneity of the measured analyte is not clear, and gravimetric units rather than molar units are preferred.

With regard to laboratory reports, we remain in a transition state between conventional (gravimetric) units and SI units. Quest Diagnostics is moving to SI units but continues to use selected conventional units during what has become a prolonged transition period. For your convenience, we have included a guide for interconversion of conventional and SI units, along with definitions of abbreviations used. The following table lists gravimetric and possible international unitage for a variety of laboratory tests. The conversion factor (CF) is used as follows:

gravimetric × CF = international unit
international ÷ CF = gravimetric unit

Abbreviation	Definition
BCE	bone collagen equivalent
creat	creatinine
d	day
dL	deciliter
g	gram
h	hour
IU	international units
kat	katal (mol/s)
L	liter
mg	milligram
min	minute
mL	milliliter
mL/h	milliliter/hour
mol	mole
mmol	millimole
mIU	milli international units
ng	nanogram
pg	picogram
pmol	picomole
s	second
U	units
µg	micogram
µmol	micromole
µU	microunits
y	year

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
ACTH	pg/mL	0.22	pmol/L
Aldosterone, S	ng/dL	0.0277	nmol/L
Aldosterone, U	μg/24 h	2.77	nmol/d
Alkaline Phosphatase, Bone Specific	μg/L	1.0	μg/L
Alpha Subunit	ng/mL	1.0	μg/L
3α-Androstanediol Glucuronide	ng/dL	0.0213	nmol/L
Androstenedione	ng/dL	0.0349	nmol/L
Angiotensin Converting Enzyme	U/L	0.017	μKat/L
Angiotensin II	ng/L	1.0	ng/L
Apolipoprotein A1	mg/dL	0.01	g/L
Apolipoprotein B	mg/dL	0.01	g/L
Arginine Vasopressin	pg/mL	0.926	pmol/L
Atrial Natriuretic Hormone	pg/mL	1.0	ng/L
B-Type Natriuretic Peptide	pg/mL	1.0	ng/L
C-peptide, 24-h U	μg/g creat	0.0377	nmol/mmol creat
C-Peptide, S	ng/mL	0.333	nmol/L
Calcitonin	pg/mL	1.0	ng/L
Calcium, Ionized	mg/dL	0.25	mmol/L
Calcium, Total	mg/dL	0.25	mmol/L
Calcium, U	mg/24 h	0.025	mmol/d
Catecholamines, P			
Epinephrine	pg/mL	5.454	pmol/L
Norepinephrine	pg/mL	5.914	pmol/L
Dopamine	pg/mL	6.524	pmol/L
Catecholamines, U			
Epinephrine	μg/24 h	5.454	nmol/d
Norepinephrine	μg/24 h	5.914	nmol/d
Dopamine	μg/24 h	6.524	nmol/d
Epinephrine	μg/g creat	0.617	μmol/mol creat
Norepinephrine	μg/g creat	0.669	μmol/mol creat
Dopamine	μg/g creat	0.738	μmol/mol creat
Cholesterol, HDL	mg/dL	0.0259	mmol/L
Cholesterol, HDL Subclasses	mg/dL	0.0259	mmol/L
Cholesterol, LDL, Direct	mg/dL	0.0259	mmol/L
Cholesterol, Total	mg/dL	0.0259	mmol/L
Chromogranin A	ng/mL	1.0	μg/L

S, serum; U, urine; P, plasma.

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
Collagen Cross-Linked N-Telopeptide (NTx)	nmol BCE/ mmol creat	1.0	nmol BCE/ mmol creat
Collagen Type I C-Telopeptide (CTx)	pg/mL	1.0	ng/L
Corticosterone	ng/dL	0.0289	nmol/L
Corticotropin Releasing Hormone	pg/mL	1.0	ng/L
Cortisol Binding Globulin	mg/L	17.18	nmol/L
Cortisol, Free, S	µg/dL	27.6	nmol/L
Cortisol, Free, U	µg/24 h	2.76	nmol/d
Cortisol, S	µg/dL	27.6	nmol/L
Cortisone, S	µg/dL	0.0277	µmol/L
Cortisone, U	µg/24 h	2.774	mmol/d
Creatinine	mg/dL	88.4	µmol/L
	g/24 h	8.84	mmol/d
Cyclic AMP, Nephrogenous	nmol/dL	10.0	nmol/L
Cyclic AMP, P	nmol/L	1.0	nmol/L
Cyclic AMP, U	µmol/L	1.0	µmol/L
Cystatin C	mg/L	1.0	mg/L
Deoxycorticosterone	ng/dL	0.0303	nmol/L
11-Deoxycortisol	ng/dL	0.0289	nmol/L
Deoxypyridinoline, Free	nmol/mg creat	113	nmol/mmol creat
Dexamethasone	ng/dL	25.48	pmol/L
DHEA	ng/dL	0.0347	nmol/L
DHEA Sulfate	µg/dL	0.0271	µmol/L
Dihydrotestosterone	ng/dL	0.0344	nmol/L
Dihydrotestosterone, Free	pg/mL	3.440	pmol/L
Estradiol, Bioavailable	pg/mL	3.67	pmol/L
Estradiol (E_2), Free	pg/mL	3.67	pmol/L
Estradiol (E_2), S	pg/mL	3.67	pmol/L
Estradiol (E_2), U	µg/g creat	0.415	nmol/mmol creat
Estrogen, Total, S	pg/mL	1.0	ng/L
Estrogen, Total, U	µg/g creat	0.113	µg/mmol creat
Estrogens, Fractionated, S			
Estrone	pg/mL	3.699	pmol/L
Estradiol	pg/mL	3.67	pmol/L
Estriol	ng/mL	3.47	nmol/L

S, serum; U, urine; P, plasma.

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
Estrogens, Fractionated, U			
Estrone	μg/g creat	0.418	nmol/mmol creat
Estradiol	μg/g creat	0.415	nmol/mmol creat
Estriol	μg/g creat	0.3925	nmol/mmol creat
Total	μg/g creat	0.113	μg/mmol
Estrone (E_1), S	pg/mL	3.699	pmol/L
Estrone Sulfate	pg/mL	2.730	pmol/L
Estrone (E_1), U	μg/g creat	0.418	nmol/mmol creat
Fructosamine	μmol/L	1.0	μmol/L
FSH	mIU/mL	1.0	IU/L
GAD-65 Antibodies	U/mL	1.0	kU/L
Gastrin	pg/mL	1.0	ng/L
Glucagon	pg/mL	1.0	ng/L
Glucose, S	mg/dL	0.0555	mmol/L
Growth Hormone	ng/mL	1.0	μg/L
Growth Hormone Binding Protein	pmol/L	1.0	pmol/L
Growth Hormone Releasing Hormone	pg/mL	1.0	ng/L
hCG, Beta Subunit	mIU/mL	1.0	IU/L
hCG, Total	mIU/mL	1.0	IU/L
5-HIAA	mg/24 h	5.23	μmol/d
	mg/g creat	0.592	μmol/mmol
Histamine			
Plasma or whole blood	ng/mL	8.997	nmol/L
Urine	μg/g creat	1.018	nmol/mmol creat
Homovanillic Acid	mg/24 h	5.49	μmol/d
	mg/g creat	0.621	μmol/mmol creat
17-Hydroxycorticosteroids	mg/24 h	2.76	μmol/d
	mg/g creat	0.312	mmol/mol creat
18-Hydroxycorticosterone	ng/dL	0.0276	nmol/L
6β-Hydroxycortisol	μg/24 h	2.642	nmol/d
18-Hydroxycortisol	μg/24 h	2.642	nmol/d
21-Hydroxylase Antibody	U/mL	1.0	kU/L
17-Hydroxypregnenolone	ng/dL	0.0301	nmol/L

S, serum; U, urine; P, plasma; GAD-65, glutamic acid decarboxylase-65.

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
17-Hydroxyprogesterone	ng/dL	0.0303	nmol/L
Hydroxyproline, Free	mg/24 h	7.63	μmol/d
	mg/dL	76.3	μmol/L
Hydroxyproline, Total	mg/24 h	0.0076	mmol/d
	mg/g creat	0.863	μmol/mmol creat
IA-2 Antibody	U/mL	1.0	kU/L
IGFBP-1	ng/mL	1.0	μg/L
IGFBP-2	ng/mL	1.0	μg/L
IGFBP-3	mg/L	1.0	mg/L
IGF-I	ng/mL	1.0	μg/L
IGF-II	ng/mL	1.0	μg/L
Inhibin A	pg/mL	1.0	ng/L
Inhibin B	pg/mL	1.0	ng/L
Insulin Antibodies	U/mL	1.0	kU/L
Insulin, Free	μU/mL	7.175	pmol/L
Insulin, Total	μU/mL	7.175	pmol/L
Invasive Trophoblast Antigen (ITA, hyperglycosylated hCG)	μg/L	1.0	μg/L
17-Ketosteroids	mg/24 h	3.47	μmol/d
	mg/g creat	0.392	μmol/mmol creat
Leptin	ng/mL	1.0	μg/L
LH (Luteinizing Hormone)	mIU/mL	1.0	IU/L
Lipoprotein (a)	mg/dL	0.01	g/L
Macroprolactin	ng/mL	1.0	μg/L
Magnesium, S	mg/dL	0.4114	mmol/L
Metanephrines, P			
Metanephrine	pg/mL	5.07	pmol/L
Normetanephrine	pg/mL	5.46	pmol/L
Total	pg/mL	5.26	pmol/L
Metanephrines, U			
Metanephrine	μg/24 h	5.07	nmol/d
Normetanephrine	μg/24 h	5.46	nmol/d
Total	μg/24 h	5.26	nmol/d
Metanephrine	μg/g creat	0.574	nmol/mmol creat
Normetanephrine	μg/g creat	0.617	nmol/mmol creat
Total	μg/g creat	0.595	nmol/mmol creat

S, serum; U, urine; P, plasma; IGFBP, IGF binding protein; IGF, insulin-like growth factor.

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
Microalbumin, U	mg/24 h	1.0	mg/d
	mg/g creat	0.113	mg/mmol creat
Osmolality, S	mOsm/kg	1.0	mmol osmotically active particles/kg H_2O
Osmolality, U	mOsm/kg	1.0	mmol osmotically active particles/kg H_2O
Osteocalcin	ng/mL	1.0	µg/L
Pancreatic Polypeptide	pg/mL	1.0	ng/L
Pepsinogen I	ng/mL	1.0	µg/L
Plasma Renin Activity (PRA)	ng/mL/h	1.0	µg/L/h
	ng/mL/h	0.2778	ng/L·s
Pregnanetriol	µg/24 h	2.97	nmol/d
	µg/g creat	0.336	µmol/mol creat
Pregnenolone	ng/dL	0.0316	nmol/L
Progesterone	ng/mL	3.18	nmol/L
Proinsulin	pg/mL	0.1134	pmol/L
Prolactin	ng/mL	0.04348	nmol/L
PTH, Biointact (amino acids 1-84)	pg/mL	0.1053	pmol/L
PTH, Intact	pg/mL	0.1061	pmol/L
PTH-Related Protein (PTH-RP)	pmol/L	1.0	pmol/L
Pyridinium Collagen Cross-Links(DPYD, PYD)	nmol/mmol creat	1.0	nmol/mmol creat
Renin, Direct	µU/mL	1.0	mU/L
Serotonin	ng/mL	5.675	nmol/L
Sex Hormone Binding Globulin	nmol/L	1.0	nmol/L
Somatostatin	pg/mL	0.611	pmol/L
T3, Free	pg/dL	0.0154	pmol/L
T3, Reverse	ng/mL	1.54	nmol/L
T3, Total	ng/dL	0.0154	nmol/L
T4, Free	ng/dL	12.87	pmol/L
T4, Total	µg/dL	12.87	nmol/L
Tartrate Resistant Acid Phosphatase (TRAP)	U/L	1.0	U/L

S, serum; U, urine; P, plasma.

International System of Units (SI Units)

Name of Analyte	Gravimetric Units	Conversion Factor	International Units
TBG	µg/mL	18.5	nmol/L
Testosterone, Bioavailable	ng/dL	0.0347	nmol/L
Testosterone, Free	pg/mL	3.47	pmol/L
Testosterone, Total	ng/dL	0.0347	nmol/L
Testosterone, Urine	µg/24 h	3.47	nmol/d
Tetrahydroaldosterone	µg/24 h	1.0	µg/d
	µg/g creat	0.113	µg/mmol creat
Thyroglobulin	ng/mL	1.0	µg/L
Thyroglobulin Antibody	IU/mL	1.0	kIU/L
Thyroid Peroxidase Antibody (Anti-TPO)	IU/mL	1.0	kIU/L
Thyroid Stimulating Hormone (TSH)	mU/L	1.0	mU/L
Thyrotropin Releasing Hormone	pg/mL	2.759	pmol/L
Triglycerides	mg/dL	0.0113	mmol/L
Vasoactive Intestinal Polypeptide	pg/mL	1.0	ng/L
Vitamin D, 1,25-Dihydroxy	pg/mL	2.400	pmol/L
Vitamin D, 25-Hydroxy	ng/mL	2.496	nmol/L
Vitamin D_2, 25-Hydroxy	ng/mL	2.42	nmol/L
Vitamin D_3, 25-Hydroxy	ng/mL	2.50	nmol/L
VMA, Urine	mg/24 h	5.05	µmol/d
	mg/g creat	0.571	mmol/mol creat

S, serum; U, urine; P, plasma; TBG, thyroxine binding globulin; VMA, vanillylmandelic acid.

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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.

†This test is performed using a kit that has not been approved or cleared by the FDA. The analytical performance characteristics of this test have been determined by Quest Diagnostics Nichols Institute. This test should not be used for diagnosis without confirmation by other medically established means.

Reflex tests are performed at an additional charge and are associated with an additional CPT code.

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