



Hormone Testing – Indications and Appropriate Use

Effective Date: May 25, 2016

Revised Date: October 27, 2022

Scope

This guideline provides recommendations on the appropriate indications for testing of selected endocrine hormones in patients aged ≥ 19 years, mainly out-patients in a primary health care setting. The document is intended to provide direction to primary care physicians and help constrain inappropriate test utilization. However, it does not represent exhaustive guidance to the appropriate use of the endocrine tests listed as other indications will be identified in the practices of Royal College of Physicians and Surgeons of Canada certified gynecologists, urologists, laboratory physicians, general surgeons performing endocrine surgery, otorhinolaryngologists, and endocrinologists.

A few of the lab tests mentioned in this guideline may be less than familiar to some practitioners, but their inclusion is intentional. A goal of completeness required listing all routinely available tests potentially relevant to a given endocrine condition. Additionally, the working group recognized that practitioners routinely vary in their chosen clinical interests, and that some may wish to pursue investigations for some conditions to a greater degree than would their colleagues. Finally, inclusion of some lesser-known tests hopefully serves to refine and advance the continuing medical education of all interested guideline readers.

The document is not intended to address the care of pediatric or transgender patients.*

Key Recommendations

- Testosterone testing in women for the investigation of low libido is not useful.
- Estradiol testing in men is not useful unless there are signs of spontaneous feminization.
- 1,25(OH)₂ Vitamin D testing, with rare exception, is indicated for the investigation of hypercalcemia with concomitantly low parathyroid hormone (PTH) or for the investigation of renal stones with high PTH and normal calcium. It is not indicated for monitoring patients receiving calcitriol.
- Insulin testing is primarily useful for investigation of spontaneous hypoglycemia or to help distinguish type I diabetes from type II diabetes, but not for establishment of insulin resistance.
- Testing to distinguish type I diabetes from type II diabetes is only useful before a patient begins taking insulin.
- The utility of salivary hormone testing in any clinical context is limited to Cushing Syndrome screening using late night salivary cortisol levels.
- Screening for growth hormone (GH) related disorders with insulin-like growth factor 1 (IGF1) should not be ordered unless there are specific symptoms of acromegaly or evidence/risk factors for hypopituitarism.

* **Note:** For information and guidance on testosterone testing for transgender people, refer to *Gender-affirming Care for Trans, Two-Spirit, and Gender Diverse Patients in BC: A Primary Care Toolkit*, produced by Trans Care BC at the Provincial Health Services Authority.

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Testing

Reproduction				
Including: Testosterone – Total; Sex Hormone Binding Globulin; Estradiol; Progesterone; Luteinizing Hormone; Follicle Stimulating Hormone; Androstenedione; Dehydroepiandrosterone; Dehydroepiandrosterone –sulphate; 17-hydroxyprogesterone; Prolactin.				
Test	Indications		Non-Indications	Notes
	Male	Female		
Testosterone – Total ¹⁻¹⁰ (T) \$15.81	Investigation of male hypogonadism. Monitoring of males receiving androgen replacement. Confirming adequacy of anti-androgen therapy in males with prostate carcinoma.	Investigation of female androgen excess.	Screening for biochemical evidence of hypogonadism is not indicated in males without symptoms. Not useful for investigation of decreased libido in females. ⁷ Not useful for monitoring females receiving androgen therapy for low libido unless overuse is suspected or unexpected virilization has developed.	For the investigation of male hypogonadism. Diurnal variation of T requires that collection be performed within 3 hours of waking. Only tandem mass spectrometry is sufficiently sensitive to accurately measure the low total testosterone seen in normal females or males rendered chemically castrate for prostate carcinoma. In BC, free testosterone is calculated by measuring total testosterone, SHBG and albumin. Refer to Appendix A: Testosterone Testing and Measurements in BC for more information. For information, refer to BCGuidelines.ca: Testosterone Testing Protocol .

Test	Indications		Non-Indications	Notes
	Male	Female		
Sex Hormone Binding Globulin (SHBG) \$13.56	Clarification of borderline low total testosterone. Investigation of males with high testosterone of endogenous origin.	Clarification of borderline high total testosterone results.		<p>In adult males (>18 yrs.), SHBG should only be performed on patients whose TT lies within the upper half of the subnormal range (approximately 3-8 nmol/L for modern assays).</p> <p>SHBG may be ordered for investigation of high total testosterone (approximately 30-35 nmol/L) in men who are not receiving androgen replacement therapy.</p> <p>Total testosterone ordered for the purposes of monitoring androgen deprivation therapy for prostate cancer, should be referred to a laboratory that performs tandem mass spectrometry with a total allowable error of less than 30% at a total testosterone concentration of 0.7 nmol/L. Alternatively, the laboratory could refer the sample to be measured by an alternate immunoassay methodology that meets this minimum standard. The total allowable error limit of 30% is in comparison to a reference method for testosterone.</p>
Estradiol² (E2) \$22.43	Not indicated in routine clinical practice. Investigation for estrogen producing neoplasms in males demonstrating spontaneous feminization including gynecomastia.	Investigation of primary ovarian insufficiency or infertility. For investigation of primary ovarian insufficiency. Where possible, order concurrently with FSH on day 3 of menstrual cycle (not day 21). Monitoring of patients receiving aromatase inhibitor therapy. Detection of estrogen-producing neoplasms. For monitoring of females undergoing fertility treatment.	Not indicated in the investigation of male osteoporosis. ¹¹ Not indicated for monitoring of men receiving testosterone therapy. ¹² Not indicated to determine if a woman is in menopause except in those with prior hysterectomy without bilateral salpingo-oophorectomy. Not indicated for monitoring of patients receiving ERT.	E2 immunoassay performance is poor at low concentration ^{2, 13} ranges making these assays inadequate for use in females receiving aromatase inhibitors ¹⁴ and in most male patients. E2 analysis by tandem mass spectrometry can be arranged for females receiving aromatase inhibitors. For the investigations of female osteoporosis, see BCGuidelines.ca: Osteoporosis: Diagnosis, Treatment and Fracture Prevention .

Test	Indications		Non-Indications	Notes
	Male	Female		
Progesterone \$14.86	Not indicated.	Investigation of infertility. Identification of ovulation. Investigation of pregnancy viability. ¹⁵	Not indicated to determine if a woman is in menopause except in those with prior hysterectomy without bilateral salpingo-oophrectomy.	Fertility measurement is made at day 21 or mid-luteal phase.
Luteinizing Hormone^{16, 17} (LH) \$12.41	Investigation of primary or secondary hypogonadism.	Investigation of primary or secondary hypogonadism. Investigation of infertility. Prediction of ovulation (by LH surge).	LH / FSH ratio is not recommended to identify women with PCOS. ¹⁸ Not indicated to determine if a woman is in menopause except in those with prior hysterectomy without bilateral salpingo-oophrectomy.	Usually performed in combination with measurement of appropriate sex steroids (T for males, E2 for females).
Follicle Stimulating Hormone^{16, 17} (FSH) \$13.13	Investigation of primary or secondary hypogonadism. Investigation of oligospermia or azoospermia.	Investigation of primary or secondary hypogonadism. Investigation of infertility. Investigation of primary ovarian insufficiency or infertility. Where possible, order concurrently with E2 on day 3 of menstrual cycle.	LH / FSH ratio is not recommended to identify women with PCOS. ¹⁸ Not indicated to determine if a woman is in menopause except in those with prior hysterectomy without bilateral salpingo-oophrectomy.	Usually performed in combination with measurement of appropriate sex steroids (T for males, E2 for females).
Androstenedione \$36.09	Not indicated in routine clinical practice. May be useful in monitoring therapy in patients with established CAH. ¹⁹	Not indicated in routine clinical practice. Functions as a second-line tool in the investigation of PCOS often in specialty practice. May be useful in monitoring therapy in patients with established CAH. ¹⁹	Not indicated in the investigation of male hypogonadism as this is an adrenal steroid.	
Dehydroepiandrosterone (DHEA) \$18.55	Not indicated.		Not indicated in the investigation of male hypogonadism as this is an adrenal steroid.	
Dehydroepiandrosterone-sulphate²⁰ (DHEA-S) \$18.55	Not indicated in routine clinical practice. Investigation of adrenal carcinoma.	Investigation of PCOS. Investigation of adrenal carcinoma.	Not useful for the investigation of male hypogonadism as this is an adrenal steroid.	
17-hydroxyprogesterone (17-OHP) \$41.71	Not indicated in routine clinical practice.	For the diagnosis of non-classic CAH as a cause of hirsutism and for monitoring of CAH therapy.		
Prolactin \$13.49	Investigation of infertility / hypogonadism / amenorrhea. Investigation of PCOS in females. Investigation of galactorrhea. Investigation for prolactinoma and other pituitary tumors.		Not useful for monitoring pregnant or lactating patients.	

Calcium and Bone Metabolism

Including: 25-hydroxyvitamin D; 1,25 dihydroxyvitamin D; Parathyroid Hormone

Test	Indications	Non-Indications	Notes
25-hydroxyvitamin D (25(OH)D) \$61.32	Investigation of vitamin D nutritional status only by specialists in clinical scenarios where biochemical identification of vitamin D deficiency is necessary.	Not useful in the investigation of hypercalcemia unless there is clinical suspicion of vitamin D supplement overdose.	Not intended for other metabolites of Vitamin D. Payable only for beneficiaries under the age of 19 years or when requested by a specialist.
1,25 dihydroxyvitamin D (1,25(OH) ₂ D) \$94.49	Investigation of hypercalcemia with concomitantly low PTH.	Should not be used for the investigation of vitamin D nutritional status. Should not be used to monitor levels of pharmaceutically administered calcitriol.	Refer to BCGuidelines.ca: Vitamin D Testing Protocol for additional information. There is some evidence Vitamin D may boost immune function. ²² As it is unlikely a patient develop vitamin D toxicity at recommended dosing, it is advisable to encourage supplementation rather than testing.
Parathyroid Hormone (PTH) \$17.52	Investigation of hyper and hypocalcemia. Monitoring and treatment of renal osteodystrophy.		In patients with previously identified hypo or hypercalcemia, measure concomitantly with a repeat total or ionized calcium.

Adrenal

Including: Aldosterone (Plasma and Urine); Renin; Cortisol (24-hour urine free); Cortisol (serum/plasma); Cortisol (late night salivary); Adrenocorticotrophic hormone stimulation test; Adrenocorticotrophic; 24-hour urinary excretion of catecholamines and metanephrines; Plasma Catecholamines; Plasma Free Metanephrines

Test	Indications	Non-Indications	Notes
Aldosterone , Plasma and Urine \$170.92	Investigation of secondary causes of hypertension: primary aldosteronism, renal artery stenosis.	Not indicated for the initial investigation of syncope or hypotension caused by possible adrenal insufficiency. For adrenal insufficiency, screen with am cortisol and proceed to 250 mcg ACTH stimulation test as indicated.	For ambulatory patients meeting criteria for primary aldosteronism screening, order upright plasma aldosterone and plasma renin activity after 1-2 hours ambulation and before 10 am. ²¹ Numerous physiological states and medications interfere with sensitivity and specificity of screening, including RAS inhibitors. ²¹
Renin \$63.87 – \$98.11	Investigation of secondary causes of hypertension: primary aldosteronism and renal artery stenosis. Monitoring of CAH and mineralocorticoid supplementation in Addison's Disease.		
Cortisol , 24-hour urine free \$26.01	Used to screen for Cushing Syndrome caused by endogenous cortisol excess only.	Not useful for the diagnosis of Addison's Disease. Do not order in patients receiving exogenous glucocorticoids.	Numerous studies have found males to have higher excretion rates than females. Application of sex specific reference intervals advised in cases where elevation is equivocal. ^{22, 23}
Cortisol , serum/plasma \$13.28	Used in the screening and diagnosis of both Addison's Disease (primary adrenal insufficiency) and Cushing Syndrome.	Not useful in the investigation of non-specific fatigue and lethargy unless accompanied by clinical syndromes of Addison's Disease or Cushing Syndrome. Measurement of morning and afternoon cortisol (for diurnal variation) is not useful.	A morning cortisol measurement can be used to exclude adrenal insufficiency. Collect sample within 3 hours of waking. Do not order random cortisol levels for this purpose. A morning serum/plasma cortisol is not useful as a screen for Cushing Syndrome. Use 1 mg overnight dexamethasone suppression, 24-hour urinary free cortisol or late-night salivary cortisol. ^{25, 26} Please note on the requisition that patient has been given 1 mg dexamethasone and provide the time of administration. The dose should be given as close to midnight as possible. For cortisol analysis after ACTH stimulation, see adrenocorticotrophic hormone stimulation test. Measurement of cortisol in patients being treated with exogenous glucocorticoids presents numerous bioanalytical and interpretive challenges and is not advised without consultation with an endocrinologist's and/or laboratory physician/scientist.

Test	Indications	Non-Indications	Notes
Cortisol, late night salivary \$77.25	Used to screen for Cushing Syndrome caused by endogenous cortisol excess only.		Restricted to Vancouver General Hospital. Payable only when requested by General Internists, Endocrinologists, Pediatricians and General Surgeons. The daily maximum is one per patient.
Adrenocorticotrophic hormone stimulation test (ACTH stimulation test) \$45.24	Used in the diagnosis of Addison's Disease (primary adrenal insufficiency) and secondary adrenal insufficiency. Used in the diagnosis of non-classic CAH.		Patients with Addison's Disease have marked elevations of plasma ACTH at initial presentation provided they are not exposed to exogenous glucocorticoid. 17-OHP is also ordered if non-classic CAH is a diagnostic consideration. Measurement of cortisol in patients being treated with exogenous glucocorticoids presents numerous bioanalytical and interpretive challenges and is not advised without consultation with an endocrinologist's and/or laboratory physician/scientist.
Adrenocorticotrophic (ACTH plasma) \$36.57	Used in the diagnosis Addison's Disease (primary adrenal insufficiency) and secondary adrenal insufficiency. Used to distinguish between Cushing Syndrome caused by adrenal adenoma and Cushing Syndrome caused by inappropriate ACTH production from pituitary adenoma or ectopic source. Used in the diagnosis of non-classic CAH.		When ACTH is measured, a concurrent measurement of cortisol should be made.
24-hour urinary excretion of catecholamines and metanephrines \$46.45 catecholamines \$155.77 Metanephrines	Used in the diagnosis of pheochromocytoma and functional paraganglioma.	Urinary vanillylmandelic acid, although the end metabolite of both epinephrine and norepinephrine, has test characteristics inferior to urinary catecholamines and metanephrines and is not required for the investigation of pheochromocytoma. ²⁷ Urinary dopamine and homovanillic acid are not required for the initial investigation of pheochromocytoma. This test is not to be used as a tool for psychiatric assessment.	This test is subject to false positives from physiological stressors, certain foods, and numerous medications. Appropriate patient preparation is required to yield meaningful results. ^{28,29} For more information, please see Catecholamines in Urine .

Test	Indications	Non-Indications	Notes
Plasma Catecholamines	This test is no longer offered in BC. Please see plasma free metanephrines.		
Plasma Free Metanephrines	Used in the diagnosis of pheochromocytoma and functional paraganglioma.	This test is not to be used as a tool for psychiatric assessment.	Not currently offered in BC. Can be arranged as an out of province send-out where required (e.g., for diagnostic clarity in ambiguous cases or for the oliguric patient). This test is sensitive to patient posture and supine collections are recommended. ²⁸

Thyroid

Including: Thyroid Stimulating Hormone; Free Thyroxine; Free Triiodothyronine; Total thyroxine/ triiodothyronine; Anti-thyroid peroxidase; Thyroglobulin/Antithyroglobulin; Antibodies to the thyroid stimulating hormone receptor

Test	Indications	Non-Indications	Notes
Thyroid Stimulating Hormone (TSH) \$9.90	Used to screen for all causes of primary hypothyroidism and hyperthyroidism. Monitoring of patients treated with thyroid hormone.	In isolation, TSH is not useful with the investigation of secondary (also known as central or pituitary) hypothyroidism. If secondary hypothyroidism is suspected, measure fT4.	TSH is the preferred test for the initial investigation of thyroid disease and for monitoring thyroid hormone replacement therapy. For the initial diagnosis of thyroid disease, confirmation of an abnormal TSH with a free T4 is indicated. Refer to GPAC Guideline: <i>Thyroid Function Testing in the Diagnosis and Monitoring of Thyroid Function Disorder (October 2018)</i> for other situations and additional information.
Free Thyroxine (fT4) \$12.12	Used for diagnostic confirmation of hyper/hypothyroidism when TSH is abnormal. Used to assess the severity of hyperthyroidism and ongoing management of Graves' Disease and other forms of hyperthyroidism. Used to monitor T4 supplementation in patients with secondary hypothyroidism (pituitary cause).	Not for use in an initial screen for thyroid dysfunction except in the unusual circumstance that there is specific reason to suspect pituitary disease.	
Free Triiodothyronine (fT3) \$9.35	Rarely indicated. Reserved for situations where hyperthyroidism is suspected clinically and TSH is suppressed, but fT4 is not elevated.	Not for use in an initial screen for thyroid dysfunction.	
Total thyroxine/ triiodothyronine (Total T4/T3)	Not currently offered by any lab in BC. Replaced by free hormone determination.		
Thyropoxidase antibodies \$20.22	Used for the diagnosis of Hashimoto's thyroiditis in the investigation of primary hypothyroidism.	Serial measurements are not indicated.	Payable only for possible autoimmune thyroid disease.
Thyroglobulin/ Antithyroglobulin (Tg/Anti Tg) \$27.90/\$20.40	Serves as a tumor marker for patients who have undergone previous treatment for papillary or follicular thyroid carcinoma.	Anti-Tg measurements are not indicated for the investigation for general thyroid autoimmunity. Tg measurement is not indicated in patients with an intact thyroid except in rare circumstances.	Anti-Tg must be measured concomitantly with Tg as the presence of Anti-Tg makes Tg results unreliable.

Test	Indications	Non-Indications	Notes
<p>Antibodies to the thyroid stimulating hormone receptor (TRAb)</p> <p>\$22.48</p>	<p>Used for the diagnosis of Graves' Disease.</p> <p>In certain clinical scenarios TRAb testing may be used as a first-line tool to distinguish Graves' Disease from other forms of hyperthyroidism. See notes.</p>	<p>Not indicated for the investigation for general thyroid autoimmunity.</p>	<p>Although radioactive iodine uptake remains the gold standard for the identification of hyperthyroidism caused by Graves' Disease, TRAb offers advantages in specific clinical scenarios: investigation of unilateral exophthalmos, euthyroid Graves ophthalmopathy, diagnosis of Graves' Disease where exposure to radioactive iodine is contraindicated (pregnancy) or where the patient is apprehensive about exposure to radiotracers. TRAb also serves as a prognostic tool in several clinical scenarios.^{30, 31} TRAb testing is more easily arranged, and results are typically available sooner than radioactive iodine uptake and scan.</p>

Growth

Including: Growth Hormone; Insulin-like Growth Factor 1

Test	Indications	Non-Indications	Notes
Growth Hormone (Somatotropin/GH) \$30.38	Used to confirm diagnosis and to assess response to treatment in acromegaly by use of dynamic function tests. Used to assess pituitary ability to generate GH in dynamic function tests for hypopituitarism or pituitary growth hormone deficiency.	A random GH should not be ordered to screen for the investigation of either acromegaly or GH deficiency.	
Insulin-like Growth Factor 1 (IGF1) \$55.08	Used to screen for acromegaly and growth hormone deficiency. Used for the monitoring response to treatment (surgical or medical) for acromegaly. Used to monitor response to therapy in patients receiving recombinant GH.	Should not be ordered unless there are specific symptoms of acromegaly or evidence/risk factors for hypopituitarism. ³²	Reference intervals are highly dependent on age. Please use age-specific reference interval provided on the lab report. A complete chart of reference intervals by age is available from St. Paul's Hospital Department of Pathology and Laboratory Medicine, at website: providencelaboratory.com .

Glucose Homeostasis

Including: Insulin; C-peptide

Test	Indications		Non-Indications	Notes
	Male	Female		
Insulin \$27.55	Investigation of hypoglycemia. Note that the specimen collected at the time of hypoglycemia must be analyzed.	Investigation of hypoglycemia. Note that the specimen collected at the time of hypoglycemia must be analyzed. Fasting insulin may be useful in the investigation of PCOS. ³³	Not indicated for patients receiving insulin therapy. Not for investigation impaired fasting glucose. Lack of standardization in insulin assays and lack of data for normative responses mean that glucose-stimulated insulin measurements are not recommended in clinical practice. ^{34, 35}	Reference intervals are BMI-dependent and require fasting state. May be helpful in distinguishing type I from type II diabetics in equivocal cases, prior to commencement of therapy. For investigation of hypoglycemia, the specimen demonstrating hypoglycemia is required and must be frozen at the earliest possible opportunity. Occasionally confirmation of surreptitious or accidental insulin administration is clinically indicated in cases of hypoglycemia. However, many insulin assays do not detect synthetic analog insulins well. Specialized analysis is required to detect synthetic analogues. Consult with laboratory physician or scientist for advice.
C-peptide \$47.42	Investigation of hypoglycemia.			Reference intervals are BMI-dependent and require fasting state. May be helpful in distinguishing type I from type II diabetics in equivocal cases, prior to commencement of therapy. C-peptide clearance is poor in patients with CKD, where large elevations above the reference interval are common.

Abbreviations: 1,25(OH)2D = 1,25 dihydroxyvitamin D; 17-OHP = 17-hydroxyprogesterone; 25(OH)D = 25-hydroxyvitamin D; ACTH = adrenocorticotrophic hormone; Anti-TPO = Anti-thyroid peroxidase; BMI = body mass index; CAH = congenital adrenal hyperplasia; DHEA = dehydroepiandrosterone; DHEA-S = dehydroepiandrosterone-sulphate; E2 = estradiol; ERT = estrogen replacement therapy; FSH = follicle stimulating hormone; fT3 = free triiodothyronine; fT4 = free thyroxine; GH = growth hormone; h = hour; IGF1 = insulin-like growth factor 1; LH = luteinizing hormone; PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin; T = testosterone-total; T4 = thyroxine; T3 = triiodothyronine; Tg = thyroglobin; TRAb = TSH Receptor Antibody; TSH = thyroid stimulating hormone.

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Appendices

- [Appendix A: Testosterone Testing and Measurements in BC](#)

Associated Documents

The following documents accompany this guideline:

- [BCGuidelines.ca: Testosterone Testing Protocol](#)
- [BCGuidelines.ca: Osteoporosis: Diagnosis, Treatment and Fracture Prevention](#)
- [BCGuidelines.ca: Vitamin D Testing Protocol](#)
- [BCGuidelines.ca: Thyroid Function Tests: Diagnoses and Monitoring of Thyroid Function Disorders in Adults](#)

This guideline is based on scientific evidence current as of the effective date.

This guideline was developed by the Guidelines and Protocols Advisory Committee in collaboration with the Provincial Laboratory Medicine Services, and adopted under the *Medical Services Act* and the *Laboratory Services Act*.

For more information about how BC Guidelines are developed, refer to the GPAC Handbook available at BCGuidelines.ca: [GPAC Handbook](#).

THE GUIDELINES AND PROTOCOLS ADVISORY COMMITTEE

The principles of the Guidelines and Protocols Advisory Committee are to:

- encourage appropriate responses to common medical situations
- recommend actions that are sufficient and efficient, neither excessive nor deficient
- permit exceptions when justified by clinical circumstances

Contact Information:

Guidelines and Protocols Advisory Committee
PO Box 9642 STN PROV GOVT
Victoria BC V8W 9P1

Email: hth.guidelines@gov.bc.ca

Website: www.BCGuidelines.ca

Disclaimer

The Clinical Practice Guidelines (the “Guidelines”) have been developed by the Guidelines and Protocols Advisory Committee on behalf of the Medical Services Commission. The Guidelines are intended to give an understanding of a clinical problem, and outline one or more preferred approaches to the investigation and management of the problem. The Guidelines are not intended as a substitute for the advice or professional judgment of a health care professional, nor are they intended to be the only approach to the management of clinical problem. **We cannot respond to patients or patient advocates requesting advice on issues related to medical conditions. If you need medical advice, please contact a health care professional.**



Appendix A: Testosterone Testing and Measurements in BC

Testosterone exists in multiple compartments in the patient plasma. Owing to its non-polar chemical structure, only a very small fraction of testosterone is freely dissolved, and this is the biologically active fraction. Testosterone is also heavily bound to two plasma proteins: (1) albumin which is high-capacity (i.e., high in concentration) and low-affinity (i.e., loosely binding) and (2) sex hormone binding globulin (SHBG) which is low-capacity (low in concentration) and high-affinity (avidly binding). Pardridge¹ hypothesized that both the albumin-bound and the free testosterone were relatively biologically available compared to the SHBG-bound testosterone. He popularized the measurement of so-called “bioavailable testosterone” which is the sum of the free and albumin-bound fractions of testosterone. With these definitions in mind, testosterone can be measured as follows:

Total Testosterone: The sum of all testosterone fractions in the patient plasma, whether free or protein bound. In BC this is routinely measured by automated immunoassay at most laboratories and by tandem mass spectrometry at St. Paul’s Hospital.

Bioavailable Testosterone: The sum of free testosterone and albumin-bound testosterone. This can be measured by selectively precipitating SHBG and performing a total testosterone assay on the remaining solution (“supernatant”). Due to poor repeatability, this method is no-longer employed in BC. Alternatively, bioavailable testosterone can be calculated from the total testosterone, SHBG and albumin concentrations using one of several formulas,² most often Vermeulen’s equation.³ Calculating bioavailable testosterone is probably a superior approach to the method of selective precipitation but is hampered by the fact that different kits for measuring total testosterone and SHBG produce different numerical results on the same patient sample. This leads to poor inter-laboratory comparability for this test. Consequently, each lab must generate reference intervals specific to their methods for total testosterone and SHBG. Given the fact that free and bioavailable testosterone demonstrate more age-dependence in older males than total testosterone,^{4,5} many subjects are required to accomplish this.

Free Testosterone: This is the testosterone that is freely dissolved in the patient plasma. In BC, this is performed by measuring total testosterone, SHBG, and albumin and then calculating the free testosterone in the same manner as the bioavailable testosterone is determined.² Calculated free testosterone is essentially a constant multiple of calculated bioavailable testosterone and neither offers any advantage over the other if both reference ranges are identically validated. In extenuating circumstances, free testosterone can be measured by a reference method using equilibrium-dialysis followed tandem mass spectrometry. This is not offered in Canada at the present time. Methods for free testosterone by analogue-based radioimmunoassay perform so poorly that they have been discontinued in BC and elsewhere.^{6,7,8}

Salivary Testosterone: Saliva offers an ultrafiltrate of the plasma and therefore salivary testosterone correlates with free testosterone in both men and women.^{9,10} However, tandem mass spectrometry is required to accurately quantify testosterone at the low levels seen in saliva (down to 0.005 nmol/L for women). This assay is not currently offered at any Canadian reference laboratory. However, results depend on the manner in which saliva is collected and are not clinically equivalent to free testosterone.¹⁰ For this reason, salivary testosterone is, at present, a research-level tool we do not recommend incorporating it into routine clinical practice.

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