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Special Endocrine Testing

Effective Date: May 25, 2016

Scope

This guideline provides recommendations on the appropriate indications for testing of selected endocrine hormones in patients aged ≥ 19 years, mainly in a primary health care setting. The document is intended to provide direction to primary care physicians and help constrain inappropriate test utilization particularly as it pertains to so-called wellness and "anti-aging" practices. 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 RCPSC certified gynecologists, urologists, laboratory physicians, and endocrinologists. The document is not intended to address the care of pediatric or transgender patients.

Testing

1. 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 For information about **Free Testosterone** and **Bioavailable Testosterone**, see *Appendix A: Testosterone Testing and Measurements in BC.*	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. Some symptoms of hypogonadism overlap with normal effects of aging.[3] 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 between 7 and 10 am or 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. For information, refer to BCGuidelines.ca: Testosterone Testing Protocol.
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.		Total testosterone is the first-line test for male hypogonadism.

Test*	Indications		Non-Indications	Notes
	Male	Female		
Estradiol[2] (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. Investigation of primary ovarian insufficiency—where possible, order in conjunction 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-oophrectomy. 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.
Progesterone $14.86	Not indicated.	Investigation of infertility. Identification of prior 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 but has clinical value in established cases.[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 in conjunction with E2 on day 3 of menstrual cycle.	LH / FSH ratio is not recommended to identify women with PCOS but has clinical value in established cases.[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 primarily an adrenal steroid.	

Test*	Indications		Non-Indications	Notes
	Male	Female		
Dehydroepiandrosterone (DHEA) $18.55	Not indicated.	Not indicated in the investigation of male hypogonadism as this is an adrenal steroid.		
Dehydroepiandrosterone –sulphate[20] (DHEA-S) $18.55	Not indicated in routine clinical practice. Investigation of adrenal carcinoma.	Investigation of PCOS. Investigation of adrenal mass.	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 galactorrhea. Investigation for prolactinoma and other pituitary tumours. Monitoring of prolactinoma in patients treated medically or surgically.		Not useful for monitoring pregnant or lactating patients.	
2. Calcium and Bone Metabolism				
Including: 25-hydroxyvitamin D; 1,25 dihydroxyvitamin D; Parathyroid Hormone				
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.	If ordered by a family physician, the patient must pay for analysis. For information, refer to BCGuidelines.ca: Vitamin D Testing Protocol.
1,25 dihydroxyvitamin D (1,25(OH)2D) $94.49	Investigation of hypercalemia 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.	
Parathyroid Hormone (PTH) $17.52 – 25.18	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.
3. Adrenal				
Including: Aldosterone (Plasma and Urine); Renin; Cortisol (24-hour urine free); Cortisol (serum/plasma); Cortisol (late night salivary); Adrenocorticotropic hormone stimulation test; Adrenocorticotropic; 24-hour urinary excretion of catecholamines and metanephrines; Plasma Catecholamines; Plasma Free Metanephrine				
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 µg 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.[21]	

Test*	Indications	Non-Indications	Notes
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	Used for targeted screening 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 targeted 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 between 7 am and 10 am or within 3 hours of waking. Do not order random cortisol levels for this purpose. An am 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.[24, 25] For cortisol analysis after ACTH stimulation, see adrenocorticotropic hormone simulation 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 endocrinologists and/or laboratory physician/scientist.
Cortisol, late night salivary $77.25	Used to screen for Cushing Syndrome caused by endogenous cortisol excess only.		Only available through Vancouver General Hospital. Measurement of cortisol in patients being treated with exogenous glucocorticoids presents numerous bioanalytical and interpretive challenges and is not advised without consultation with an endocrinologists and/or laboratory physician/scientist.
Adrenocorticotropic 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 that 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 endocrinologists and/or laboratory physician/scientist.
Adrenocorticotropic (ACTH) $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.		For the investigation of adrenal insufficiency, concomitant am ACTH and cortisol should be performed before pursuit of ACTH stimulation testing.

Test*	Indications	Non-Indications	Notes
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.[26] 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.[27, 28]
Plasma Catecholamines $59.27	Of limited use in the diagnosis of pheochromocytoma and functional paraganglioma.	This test is not to be used as a tool for psychiatric assessment.	This test is very sensitive to stress-related fluctuations during collection and requires an indwelling catheter, supine posture and a calm environment to generate useful results.
Plasma Free Metanephrines $N/A	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.[27]
4. Thyroid			
Including: Thyroid Stimulating Hormone; Free Thyroxine; Free Triiodothyronine; Total thyroxine/ triiodothyronine; Anti-thyroid peroxidase; Thyroglobulin/ Antithyroglobulin; Antibodies to the thyroid stimulating hormone receptor			
Thyroid Stimulating Hormone (TSH) $9.90	Used to screen for all causes of primary hypothyroidism and hyperthyrodism. Monitoring of patients treated with thyroid hormone.		TSH may be repeated after at least 6–12 weeks following a change in thyroid hormone replacement dose or after a change in a patient's clinical status. TSH monitoring in pregnancy can be as frequent as every 4–6 weeks.[29] Once the TSH has normalized with treatment, it should be checked annually unless clinically indicated. For information, refer to BCGuidelines.ca: Thyroid Function Tests: Diagnoses and Monitoring of Thyroid Function Disorders in Adults.
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.	

Test*	Indications	Non-Indications	Notes
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.		
Anti-thyroid peroxidase $20.22	Used for the diagnosis of Hashimoto's thyroiditis in the investigation of primary hypothyroidism.	Serial measurements are not indicated.	
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.
Antibodies to the thyroid stimulating hormone receptor (TRAb) $22.48	Used for the diagnosis of Graves' Disease. 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.	Not indicated for the investigation for general thyroid autoimmunity.	Although radioactive iodine uptake remains the gold standard for the identification of hyperthyroidism caused Graves' Disease, TRAb offers advantages in specific clinical scenarios: investigation of unilateral exophthalmos, euthyroid Graves opthalmopathy, 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 a number of clinical scenarios.[30, 31] TRAb testing is more easily arranged and results are typically available sooner than radioactive iodine uptake and scan.
5. Growth			
Including: Growth Hormone; Insulin-like Growth Factor 1			
Growth Hormone (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 (GF1) $55.08	Used to screen for acromegaly and growth hormone deficiency in symptomatic patients. 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 hypopitutarism.[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.

6. Glucose Homeostasis			
Including: Insulin; C-peptide			
Test*	**Indications**	**Non-Indications**	**Notes**
Insulin $27.55	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 in females.[33]	Not indicated for patients receiving insulin therapy. Not for the investigation of impaired fasting glucose or the establishment of insulin resistance. 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 1 from type 2 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 1 from type 2 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 = adrenocorticotropic hormone; Anti-TPO = Anti-thyroid peroxidase; BMI = body mass index; CAH = congenital adrenal hyperplasia; CKD = chronic kidney disease; 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.

Note: *Includes the Fee-for-Service costs as per the Schedule of Fees – Laboratory Services Payment Schedule, as of October 1, 2015.

Resources

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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, approved by the British Columbia Medical Association, and adopted by the Medical Services Commission.

THE GUIDELINES AND PROTOCOLS ADVISORY COMMITTEE

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- permit exceptions when justified by clinical circumstances

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Disclaimer

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). Pardridge[1] 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 labs 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 a number of 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] a large number of subjects is 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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