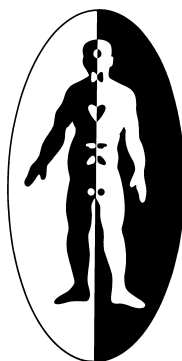


**This Handbook Is a Users Guide to the Hormone
Measurement and Consultation Service Offered By
the Hormone Laboratories of Canterbury District
Health Board at Christchurch Hospital**



**Endolab, Nuclear Medicine Laboratory
&
Canterbury Health Laboratories
Are
IANZ registered**

**This Hormone Handbook is
now available on the Internet at:**

www.cdhb.govt.nz/chlabs – Endocrinology

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Introduction

About This Booklet and Endolab

This booklet gives information regarding Endocrine and some other (non-hormone) tests available from the Christchurch Endolab group of laboratories, who offer a measurement and consultation service.

The scientists encourage technical enquiries about the tests and specialist physicians and endocrinologists are available to make clinical comment on results for both diagnosis and treatment - see page 6. Contact can be made by a toll free call: **0800 endolab (3636 522)**

The handbook includes clinical indications for the assays, sampling and transport instructions and brief notes on the interpretation of results.

Tests Offered:

Routine Tests: may be requested by filling in the Endolab request form, or any other Laboratory request form, and following the individual sampling and transport instructions provided in the alphabetical listing of tests in this handbook.

Urgent Test Results: Requests for urgent tests must be made by phone (0800 endolab (3636 522)) or fax (03 364 0818). The following information is essential:

1. When the result is required.
2. Who it is to be sent to
3. The phone/fax/pager/beeper number of the recipient.

Research Tests: Endolab laboratories can offer a service for samples from Research projects, and these requests need to be submitted to the Endolab Group for approval and budgeting prior to samples being taken. Discounts are usually available for batch or bulk processing. Payment will normally be by monthly account, but lump sum payments can result in further discounts. Please inquire about tests not listed.

Other Tests: A few tests are not offered on a routine basis but may be available after communicating directly with the laboratory concerned. Adequate justification for the test needs to be provided. Refer to page 6 for contact addresses, telephone and fax numbers.

Requisition Forms:

A sample form can be photocopied from the appendix of this handbook. Pads of forms are available free from Endolab. Any requisition form used should clearly indicate:

1. Patient details

2. Time & date of collection
3. Address for return of results
4. Address for invoicing (if appropriate)
5. Brief clinical details/drug therapy
6. Tests requested
7. Conditions of sampling (if appropriate)
8. Current drug therapy

Conditions of sampling:

Other important factors affecting the level of the hormone being assayed (see sections on individual hormones) should be noted, eg. posture or sodium content of the diet (renin and aldosterone), or whether the patient is unduly stressed (ACTH, cortisol, growth hormone) or faint and/or nauseated (AVP).

Current drug therapy eg:

1. Glucocorticoids affect ACTH and cortisol values.
2. Phenothiazines, L-dopa, metoclopramides, affect prolactin.
3. Contraceptive steroids affect LH, FSH, thyroxine and cortisol.
4. Diuretics affect renin and aldosterone.
5. Carbimazole, Iodine¹³¹, thyroid surgery should be mentioned when requesting thyroid function tests.

Sample Collection:

Check with Endolab before submitting samples that may be radioactive, that is to say from patients who have recently had a radioisotope administered to them. Such samples are not suitable for many of our tests.

Blood: It is recommended that all samples be **deep-frozen** after separation of the red cells and held at a minimum of -15°C prior to being despatched.

In general, plasma enzymes rapidly destroy peptide hormones and therefore separation of plasma and freezing of samples should be carried out within 15 minutes of collection. Samples must remain frozen until they are assayed, unless recorded as suitable for ambient transport. Badly haemolysed samples may give unreliable results and will not be assayed by the laboratory.

Never freeze whole blood as this causes severe haemolysis.

It is essential that all samples should be clearly labelled with labels, which do not come off in water. Labels must include patient's name,

initials, and the date (and time if more than one sample has been taken within 24 hours).

Whole blood-draw volumes = 2.5 times the plasma volumes, eg. **2.0ml plasma requires 5.0ml blood to be drawn.**

Please also refer to the specific instructions given in the section on individual hormones.

Urine: 24-hour collections are usually necessary, but some assays (eg. pregnanediol) may be done on random urine samples. Details regarding the volume of urine necessary and preservatives are given under individual hormones.

Please label sample with:

1. Patient's ID details
2. Collection date (eg 24h urine September 16-17 1996).
3. 24 hour urine volume (where necessary), on both the container and the request form.

The sample should be stored frozen until despatched to the Endolab.

NOTE: As it is not possible to aliquot the plasma sample after it is frozen please provide a separate frozen aliquot for each measurement requested. Considerable delays occur when a single frozen sample is sent requesting multiple measurements - especially when these are performed on different sites.

All samples including those in a series, must have individual unique lab ID numbers.

Samples are usually kept for two months

Sample Transport:

It is important that **leak-proof plastic tubes or bottles** be used for transporting samples. IATA (International Air Transport Association) approved leak proof plastic outer containers must be used for transporting samples. Regulations now exist to control the way in which specimens are transported. NZ Post will no longer accept any diagnostic specimens for delivery. IATA now regulates the packing & labelling required for air transport of specimens or infected material. Additional regulations cover the carriage of Dry Ice (solid CO₂). ESR-NZ (Environmental & Scientific Research) regulates the packing requirements for land transport of specimens &/or infectious materials. Please contact us if you need assistance with sample transport.

Infectious Samples:

Samples known to be from infectious patients (ie with HEPATITIS or AIDS) must be labelled:

INFECTIOUS

and transported inside a separate plastic specimen bag, with the form in the outer pocket. Infectious sample transport must also comply with IATA & ESR regulations.

Note: Samples from patients not known to be infectious should be labelled "Diagnostic Specimen". Do not label them "Infectious".

Ambient Samples: It is recommended that leak-proof plastic tubes are used and wrapped in bubble-wrap with sufficient absorbent material. Ambient samples must reach the laboratory within five days of sampling.

Frozen samples: Where deep frozen samples are specified, a polystyrene box or equivalent type of insulated container should be used together with an outer cardboard box. Dry Ice (solid CO₂) is an ideal medium, but is regarded as a hazardous substance when transported by air, and packages must be labelled to IATA regulations to indicate its presence. Alternatively, use Biofreeze BioBottles (www.bio-bottle.com) available from Dangerous Goods Management, Auckland Airport. Be sure that paper-labelled tubes do not come into direct contact with ice cubes and the outer container is watertight.

The use of pure water ice cubes, domestic Slikka/Polar packs and such like has proved to be unsatisfactory.

There is no need to prepare separate parcels for each laboratory as the contents are easily and rapidly distributed between laboratories.

Return of Results:

Please provide an address for the return of results. Endolab results are available via the Canterbury Health Labs Delphic reporting system. Urgent results will be telephoned or sent via Facsimile to the number indicated on the requisition form. The requestor must contact the laboratory at the time of sample collection to confirm the degree of urgency.

Reference Intervals:

Current reference intervals will be provided on all result forms. These may be subject to variation differentiated by age and sex where important/available. They are generally based on a reference population derived from the Christchurch electoral rolls and include 95% of the "healthy" volunteers. It is emphasised that the reference intervals for hormones and results published by different laboratories are NOT DIRECTLY COMPARABLE as these depend on the hormone standards, antisera and methods used.

Comparability:

Hormone test results produced by different laboratories using different methods are usually not directly comparable because methodological differences, particularly in antiserum specificity, can greatly alter the estimate of the concentration of a hormone. A laboratory's results can only be interpreted using the reference (normal) ranges issued by that laboratory.

Confidence Limits:

Some results are reported with an analytical uncertainty. This is a 95% confidence interval and refers to measurement error, not the reference interval. The true measurement error may be larger than that calculated.

Note that occasionally values that have been read off the lower end of an RIA standard curve may have an upper 95% confidence limit of infinity. In these cases the algorithm for calculating analytical uncertainty will give a value of the order of 4000. Thus very large analytical uncertainties are not to be taken literally, but as an indication that that particular result has no upper 95% confidence limit.

SI Units: where the molecular weight of a hormone is accurately known (eg steroid and thyroid hormones, insulin, C-peptide) results are expressed in SI units of amount (moles per litre). Other peptide hormones are reported in terms of the appropriate International Reference Preparation (by Courtesy of the Division of Biological Standards, National Institute for Medical Research, London) in gravimetric or biological activity units per litre.

Note:

milli (m) - one thousandth (10^{-3})

micro (μ) - one millionth (10^{-6})

nano (n) - one thousandth of a millionth (10^{-9})

pico (p) - one millionth of a millionth (10^{-12})

g - grams

L - litres

mol - moles

M - molar = moles/L

Warning: frequent interference in immunoassays from human anti-animal antibodies.

Where the results of measurements performed by immunoassays do not fit the clinical picture, the possibility must be considered that there is interference in the test method from human anti-animal antibodies (HAAA) circulating in the patient's plasma. There have been occasional reports over many years of this problem (recently reviewed by Ismail and Barth (2001) and by Kricka (1999), which can affect both the more recent automated two-site immunoassays as well as the older radioimmunoassays and can result in both artefactually high and artefactually low results being reported. The general impression given by the literature is that the two-site assays are more susceptible but a recent report showed that it is possible for even multiple radioimmunoassays to be affected by the plasma from one patient (Park et al. 2003).

It is thought that 30-40% of the population have circulating HAAA (Ismail and Barth 2001) and although in most cases the HAAA are not of sufficient concentration, specificity or affinity to cause noticeable interference in assay methodology, it seems that 0.5% or more of immunoassay results could be significantly affected (Ismail and Barth 2001). Serious consequences, including unnecessary chemotherapy and surgery, have on occasion followed from such spurious immunoassay results.

Whenever there appears to be a discrepancy between clinical findings and immunoassay results, the laboratory should be informed since it can usually test the specimen for the presence of interference by one or two of several methods. These include dilution, PEG extraction, and addition of non-immune animal sera or measurement by a different method.

References:

Ismail AAA, Barth JH. *BMJ* 323:706-7 (2001)

Kricka LJ. *Clin. Chem.* 45:942-56 (1999)

Park A, Edwards M, Donaldson M, Chatei M, Meeran K. *BMJ.* 326:541-2 (2003).

Quality Control

All Endolab analytical procedures contain two or more different concentrations (levels) of quality control (QC) samples in each batch. The QC values for each batch are checked for acceptability using a novel state-of-the-art quality control system (KiwiQC) that improves on the commonly used Westgard scheme by:

- Having improved power on account of being based on the control of the mean and variance rather than on the control of individual values and their ranges.
- Making appropriate statistical allowance for:
 - Start-up with a small number of preliminary batches.
 - Multiple levels of QC samples.
 - Replication of QC samples within a batch.
 - Variation in the number of replicates between levels and batches.
 - Autocorrelation arising from random effects.

Reference: Livesey JH. Mean and variance quality control for multiple correlated levels of replicated control samples. *Clin Chem Lab Med* 2005; 43: 1240-52.

Suggested Hormone Tests for Common Endocrine Disorders

Adrenal Problems:

Addison's disease

Synacthen test (preferably at 0800h), plasma ACTH, renin, aldosterone.

Adrenal suppression. (Patients on steroids)

Synacthen test, withholding morning prednisone dose until test completed. If response subnormal, hold prednisone dose at 3-4 mg mane, or hydrocortisone dose at 15 mg mane, until synacthen test, repeated at 2-3 monthly intervals, is normal.

Congenital adrenal hyperplasia

Plasma 17OH progesterone during follicular phase of cycle (if indicated, after synacthen), cortisol, plasma ACTH and renin.

Conn's syndrome

Plasma renin and aldosterone (preferably ambulant and before 10 am).

Cushing's syndrome

24-hr urine cortisol (and creatinine excretion), 1 mg overnight dexamethasone suppression test, plasma ACTH for differential diagnosis once elevated 24h urine cortisol is confirmed (other tests, call (03) 364 0927).

Phaeochromocytoma

24 hour urine (in acid) for catecholamines and metanephrines.

Virilising disorder

Plasma testosterone, SHBG, DHEA sulphate, 24h urine cortisol (if indicated).

Calcium problems:

Hypocalcaemia

Plasma parathyroid hormone, 25(OH) - Vitamin D, magnesium.

Hypercalcaemia

Parathyroid hormone (if normal, PTH related peptide, 25(OH) - Vitamin D).

Paget's disease, increased bone turnover

Fasting (8-9am) plasma β -CTX and bone specific alkaline phosphatase.

Glucose (Islet cell) problems

Hypoglycaemia

Plasma insulin (after overnight fast or during hypoglycaemic episode, **measure glucose on the same sample as insulin**). C-Peptide measurement will distinguish between endogenous and exogenous sources of insulin.

Gonadal problems:

(1) Females

Amenorrhoea/Menstrual Disturbance

LH, FSH, prolactin, oestradiol, free thyroxine index and TSH. Tests for hirsutism if indicated. Fasting glucose and lipids if polycystic ovary syndrome is suspected.

Menopause

Raised LH & FSH, low oestradiol may indicate that the patient is perimenopausal. Considerable fluctuations may be seen over many months until the patient is truly menopausal. Patients receiving HRT will show a fall in LH and FSH, but symptoms and bone density are more relevant parameters to monitor.

Hirsutism

Basic screening: plasma testosterone, sex hormone binding globulin.

Additional tests: LH, FSH, Adrenal androgens (eg DHEA-S), 17OH Progesterone.

Tests for Cushing's or acromegaly if indicated.

Infertility

Tests for ovulation: Plasma progesterone, one or more samples between days 21-24 of the cycle. (First day of menstruation = day 1). For other tests, see Amenorrhoea and Hirsutism.

Ovulation profile (page 79).

Polycystic Ovary Syndrome (PCOS)

Basic screening: Testosterone, Prolactin, TSH, T4, fasting lipids, fasting glucose.

75g oral GTT if:

(1) fasting glucose >5.5mmol/L

(2) obese BMI >30kg/m²

(3) family history of type 2 diabetes in 1st degree relative

Consider 17-OH progesterone, DHEAS, urine cortisol or 1mg Dexamethasone suppression test if clinically indicated.

(2) Males

Gynaecomastia

Plasma oestradiol, LH, FSH, prolactin, testosterone, sex hormone binding globulin, β -HCG. Consider TSH if symptoms of thyrotoxicosis.

Hypogonadism

Morning testosterone, SHBG, LH, FSH, prolactin.

Impotence

Testosterone, sex hormone binding globulin, prolactin, LH, FSH.

Heart Problems

Heart failure

proBNP (brain natriuretic peptide).

Hypertensive problems

See Conn's syndrome and Pheochromocytoma.

Pituitary problems

Hypopituitarism

Free thyroxine index, LH, FSH, prolactin, oestradiol, testosterone, plasma cortisol (8 am), synacthen test, plasma IGF-1.

Galactorrhoea/Amenorrhoea

Plasma prolactin. If elevated, confirm with repeat sample.

Cushing's disease

See Adrenal Problems, Cushing's syndrome.

Acromegaly

Plasma IGF-1. If IGF-1 is raised, proceed to glucose suppression, 75g oral glucose tolerance test (GH at 0, 30, 60, 90 and 120 minutes).

Short/tall Stature

Plasma IGF-1, IGFBP-3, free thyroxine index, TSH and coeliac antibodies.

Short stature: plasma growth hormone during stimulatory tests (Clonidine, Hypoglycaemia, Sleep, Exercise, Arginine. Refer to Index, page 118 for details).

Thyroid problems

Hypothyroidism

Free thyroxine index, TSH.

Hyperthyroidism

Free thyroxine index, TSH, triiodothyronine.

Please note some hormones, eg ACTH, PRA, catecholamines require special collection conditions. The nurses in the Endocrine Test Centre, Ward 26, Second Floor, Riverside Block, Christchurch Hospital will ensure that special test procedures and sampling are carried out correctly.

Telephone (03) 364 0934

Free Phone 0800 endolab (3636 522)

Fax (03) 3640 1159

Tests in Alphabetical Order

11-Deoxycortisol

Clinical Applications:

Metyrapone can be used to provide a short test of the integrity of the pituitary-adrenal axis. 30mg/kg metyrapone is given at midnight and blood sample is drawn at 0800 hr the following morning for cortisol and 11-deoxycortisol assay. (See also Metyrapone Test, page 102 for precautions). There is a good correlation between a normal metyrapone response and full integrity of the hypothalamic-pituitary-adrenal axis. Serum levels of 11-deoxycortisol are elevated in patients with adrenal 11- β -hydroxylase deficiency.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	
Volume	Basal, 1ml serum/plasma, 250 μ l serum or plasma minimum. Post metyrapone, 1ml serum/plasma. Request MUST state whether sample basal or post-metyrapone, since different dilutions are used.
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	On demand
Reference Interval:	basal, < 30nmol/L post metyrapone, > 200nmol/L
Standard:	
Method:	ELISA

ACTH, Adrenocorticotrophic Hormone, Corticotropin

Clinical Applications:

1. Aetiology of Cushing's Syndrome. (Note that ACTH determinations are not useful in establishing the diagnosis of Cushing's Syndrome.)
2. Plasma ACTH levels taken during bilateral inferior petrosal sinus sampling will help to distinguish pituitary-based Cushing's disease from the ectopic ACTH syndrome. Lateralisation of the tumour is also often possible.
3. The ACTH response to corticotrophin releasing factor (CRF) is useful in distinguishing between pituitary-based Cushing's disease (enhanced response) and ectopic ACTH syndrome (usually an absent response).
4. Both the above procedures should be carried out in consultation with an endocrinologist.
5. Assessment of the response to therapy of Cushing's Syndrome
6. The response to pituitary ablation in Nelson's Syndrome and pituitary-based Cushing's disease.
7. The response to surgery or radiotherapy in the ectopic ACTH Syndrome.
8. The early detection of an ACTH secreting pituitary tumour of Nelson's Syndrome, following bilateral adrenalectomy.
9. Diagnosis of primary adrenal insufficiency whether due to Addison's Disease or an adrenal enzyme defect.
10. Assessment of the adequacy of replacement therapy in primary adrenal insufficiency.
11. Assessment of hypothalamic-pituitary function. (Note that resting levels will not distinguish normal from impaired secretion. Dynamic tests of ACTH release such as insulin induced hypoglycaemia or metyrapone are necessary. In such cases the plasma cortisol response to hypoglycaemia and the plasma 11-deoxycortisol response to metyrapone will give the desired information more rapidly and cheaply).

Patient Preparation

Patient - need not be fasting but should be unstressed. A difficult venepuncture may cause increased ACTH secretion. Time of sampling must be stated (because of the diurnal variation in ACTH values) and the time of last dose of glucocorticoid (where relevant). In patients with Cushing's Syndrome it may be useful to take samples at 0900 hr and 2400 hr. Insulin hypoglycaemia or metyrapone testing are necessary when assessing hypothalamic - pituitary function (see above 11).

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	1ml EDTA plasma. (0.5ml EDTA minimum).
	Sample must be immediately separated in a refrigerated centrifuge and the plasma placed in -20°C freezer within 1 hour of venepuncture. If this is not possible see options below:
	Send the patient to the Endocrine Test Centre for sample taking (7.30 am to 12 noon, Mon - Fri, telephone/appointments extension 80934) - all other blood samples can be collected at the same time.
	Or
	The sample can be collected into an EDTA tube, cooled to 0°C (by immersion into water-ice mixture) where it can be held for up to thirty minutes, and then separated and placed in a -20°C freezer within 1 hour.
	Note ACTH is rapidly destroyed by plasma enzymes - serum, heparin and fluoride samples are not acceptable as results are lowered. Warm thawed samples may not be assayed. Thawing and re-freezing the sample may elevate the levels.
Transport	Frozen
Storage	Frozen
Species	Human, others not known.

Test Details:

Frequency:	Fortnightly
Reference Interval:	Adult: 1.0-12.0pmol/L (0700-1000 h)

Conversion factor: ng/ml = 4.5 x pmol/L

Standard: hACTH 1-39

Method: Roche Elecsys

Interpretation

Raised values - stress, Cushing's Syndrome, (hypothalamic-pituitary disease or ectopic ACTH secretion from a tumour), ACTH secreting pituitary tumours with hyper pigmentation, (Nelson's Syndrome), Addison's Disease, Adrenal enzyme deficiency, adrenalectomy.

Undetectable values - Cushing's Syndrome (adrenal adenoma or carcinoma) glucocorticoid therapy. Note that ACTH values may be undetectable in normal individuals, particularly in the late evening.

Aldosterone - plasma, serum

Clinical Applications:

In the diagnosis of primary aldosteronism or similar forms of hypokalaemic hypertension.

In localising the aldosterone adenoma by adrenal vein catheter sampling.

In certain types of adrenocortical insufficiency - particularly hypoaldosteronism or other salt wasting disease associated with abnormalities of steroid biosynthesis.

In cases of hypokalaemia query cause, eg Bartters syndrome

Hyporeninaemic syndromes producing hyperkalaemia.

Note: Renin measurement is indicated in most of the above applications. (See page 84)

Interpretation of adrenal vein aldosterone levels require simultaneous central (adrenal vein) and peripheral sampling for cortisol and aldosterone levels. Constant low dose ACTH stimulation may assist interpretation of results. Please consult with an Endocrinologist before undertaking adrenal vein sampling tests. Remember that prior drug use (eg. spironolactone, amiloride, ACE inhibitors, AT₂ antagonists, β blockers and diuretics) may affect interpretation of aldosterone and renin results.

Patient Preparation

Patient posture, salt intake, drug therapy, age and time of sampling affect levels. Potassium depletion and/or hypokalaemia lower aldosterone secretion.

Outpatients are best screened as follows: If possible stop non-essential drugs for two weeks before sampling. Many hypotensive drugs alter renin – aldosterone levels; preferred agents are alpha-blockers (eg. Cardoxan, Prazosin) or non-dihydropyridine calcium channel blockers (eg Verapamil) since they do not greatly alter aldosterone. Screening tests for primary hyperaldosteronism and related conditions can still be done in patients on betablockers, ACE inhibitors and/or diuretics **BUT** interpretation must allow for the potent effects these drugs have on the renin – aldosterone axis. Patients should attend (non-fasting) prior to 10.00am for “ambulant” sampling of plasma aldosterone and PRA (see page 84). It is usually wise to check plasma Na, K and creatinine at the time of sampling.

Inpatients are screened as above and should be ambulated for at least 30 minutes before sampling.

Other protocols involving plasma aldosterone PRA measurement include **saline suppression** (2L saline over 4 hours), **4-hour posture test** (08.00h overnight supine plasma aldosterone, repeated after 4 hr of upright posture) and tests using ACTH stimulation or dexamethasone suppression.

Consult with an Endocrinologist for indications and test protocols.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	0.5ml plasma (0.25ml minimum (duplicate) 0.1ml (singleton)). Blood must be centrifuged immediately, preferably at 4°C and transported deep-frozen.
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	0700 -1100 hr ambulant: 100 - 800pmol/L
Standard:	
Method:	RIA

Interpretation (refer also to aldosterone-renin ratio, see page 22)

Interpretation depends on renin status. Plasma aldosterone/PRA ratio greatly assists in diagnosis.

High plasma aldosterone levels occur in primary aldosteronism, where the aldosterone/PRA ratio is usually greater than 1000 using Endolab methods. Note that hypokalaemia could affect interpretation, since aldosterone secretion is reduced by potassium depletion. High values may also occur in secondary hyperaldosteronism, in sodium depletion and in many oedematous disorders and in these states PRA is usually elevated.

Low plasma aldosterone levels - hypoaldosteronism, hyporeninaemia syndromes, (eg non-aldosterone dependent forms of hypermineralocorticoidism), Addison's disease and some forms of congenital adrenal

hyperplasia. Low values may also occur in normal subjects receiving liberal salt intakes, and in the aged (>60 years).

Details regarding drug therapy, time of sampling, and posture are necessary for interpretation.

Age affects aldosterone levels. Values are higher in very young children and lower in subjects over 60 yrs.

Plasma aldosterone values are likely to be unreliable in severe renal failure, including patients on dialysis.

Aldosterone - urine

This test is no longer performed in New Zealand.

Aldosterone-Renin Ratio (ARR)

Clinical Applications:

In screening hypertensive patients for the presence of possible primary aldosteronism.

Patient Preparation:

The test is best done in the ambulant state between 0800-1000hrs, preferably in the absence of drugs affecting the renin – aldosterone axis. Preferred drugs (in patients unable to stop hypotensive therapy) include alpha-blockers (eg doxazosin, prazosin) or calcium channel blockers. Other hypotensives (β -blockers, ACE inhibitors or angiotensin II blockers, diuretics) may affect results (see “Interpretation”). Note that the use of spironolactone or high dose amiloride (>10 mg daily) within 6 weeks of testing will invalidate the test.

Plasma sodium, potassium and creatinine should be measured along with ARR (see below).

Sample Aliquot Requirements:

Laboratory: Endolab
Request both Aldosterone and Renin assay
Refer to page 19 for Aldosterone and page 84 for Renin respectively

Test Details:

Reference interval for aldo/renin ratio:	Suspicious	$>800 \frac{\text{pmol/L}}{\text{nmol/L/hr}}$
	Abnormal	$>1000 \frac{\text{pmol/L}}{\text{nmol/L/hr}}$

Interpretation:

Aldosterone-Renin ratio values exceeding 1000 strongly support the presence of primary aldosteronism provided plasma aldosterone exceeds 400pmol/L and the patient does not have chronic renal failure. Patients with hypokalaemia, or receiving ACE inhibitors, Ang II blockers, diuretics may have false negative results, requiring repeat tests when eukalaemic or after appropriate change in drug therapy.

NB: Definitive testing (eg saline suppression test, urine aldosterone secretion during high sodium intake) is usually required to confirm the presence of primary aldosteronism.

Alpha-subunit, Gonadotrophin

Clinical Applications:

May be of value in the detection of some tumours. Has been reported to be elevated in the plasma of a proportion of patients with pituitary or pancreatic endocrine tumours.

Patient Preparation

No special preparation. Provide brief clinical details.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA plasma
Volume	1ml plasma. Minimum 0.5ml
Transport	Ambient within 24 hours, otherwise frozen
Storage	$\leq -15^{\circ}\text{C}$
Species	

Test Details:

Frequency:	Irregular (approximately 3 monthly)
Reference Interval:	when LH/FSH/TSH/hCG are normal total: $< 3\mu\text{g/L}$. Postmenopausal, $< 20\mu\text{g/L}$ free: $< 1.4\mu\text{g/L}$ Postmenopausal, $< 5\mu\text{g/L}$
Standard:	hCG α - Biogenesis
Method:	RIA

Androstenedione - plasma, serum

Clinical Applications:

Hirsutism, virilisation.

17 Keto-reductase deficiency (rare).

Patient Preparation

No special preparation. A preference for sampling between 08.00 – 10.00 hr exists, since better comparison with normal subjects is then available. Please state time of sampling, nature of drug therapy and provide brief summary of clinical particulars.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA
Volume	0.5ml serum or EDTA plasma, minimum 0.2ml
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	<6nmol/L
Standard:	
Method:	RIA

Interpretation

Androstenedione is secreted by the ovary, the testis and the adrenal and can be converted peripherally to the active androgen, testosterone. Plasma concentrations of androstenedione are elevated in 15% of hirsute patients. Markedly elevated levels occur in patients with androgen producing adrenal or ovarian tumours and in enzyme deficient states which effect an increase in androgen production.

Angiotensin II - plasma

Clinical Applications:

This assay is still regarded as a research procedure. The clinical applications are similar to those of renin (see page 84) which should normally be requested since it is easier to measure. However, if abnormalities are suspected in the generation of Angiotensin II in vivo, (excessively rare in untreated patients), assay of Angiotensin II may be preferable to PRA. Call an Endocrinologist before testing.

Patient Preparation

Patient's posture, sodium intake, drug therapy and time of sampling all profoundly alter PRA.

Outpatients are best screened as follows:

If possible stop non-essential drugs for 2 weeks before sampling. Many hypotensive drugs alter renin levels; diuretics and ACE inhibitors increase PRA, whereas Beta-blockers reduce PRA. Alpha-blockers (Cardoxan, Prazosin and related drugs) or calcium channel blockers have less effect and are therefore preferred where the clinical condition allows.

Patients should attend (non-fasting) prior to 10.00 am for "ambulant" sampling for PRA. Plasma aldosterone (see page 19) also usually necessary for interpretation.

Inpatients are also screened as above and should be ambulated for at least 30 minutes before sampling. "Bed bound" patients may be sampled after a similar time in sitting position.

Other protocols involving Angiotensin II measurement include frusemide challenge, 2 hours of quiet standing or response to sodium depleting diets. Consult with an Endocrinologist for indications and test protocols.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	Special AII cocktail tube containing specific enzyme inhibitors including enalkiren. Endolab will supply these on request. On receipt, freeze the AII inhibitor cocktail tubes and keep for a maximum of four months.
Collection	On the day of the blood collection thaw the inhibitor cocktail tubes in hot tap water and mix well. Draw a 3ml blood sample with a 5ml syringe and needle and then transfer the blood from the syringe to the AII enzyme cocktail tube. Mix the contents gently with 4 or 5 inversions. Immediately centrifuge the tube, separate the plasma and transfer the plasma to the sample tube (labelled "AII plasma only") provided. Freeze the plasma immediately (preferably snap frozen on dry ice or liquid nitrogen). The plasma sample should then be transported frozen. Thawed samples will not be assayed.
Volume	1.1ml special plasma (volume - 3ml of blood).
Transport	Frozen (Thawed samples will not be assayed).
Storage	Frozen

Test Details:

Frequency:	This test is available only by prior consultation
Reference Interval:	6 – 24pmol/L between 7.30 – 11.30 am
Standard:	Synthetic AII (1-8)
Method:	RIA

Interpretation

Low Angiotensin II: primary aldosteronism and other mineralocorticoid excess syndromes; "low renin" hypertension; in the aged population.

High Angiotensin II: adrenocortical insufficiency, fluid and salt wasting syndromes; Bartter's syndrome, severe renal ischaemia, malignant hypertension, renin secreting tumours, and in young children.

Details regarding previous drug therapy (including oral contraceptives), date of last menstrual period and 24-hr urine electrolyte excretion will aid interpretation.

ANP, Atrial Natriuretic Peptide

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2.2ml plasma in iced water, by arrangement only
Transport	Frozen
Storage	-20°C
Species	human, ovine

Test Details:

Frequency:	By arrangement only
Reference Interval:	4-27pmol/L (human); 4.7 – 19.2pmol/L (ovine)
Standard:	ANP (99-126)
Method:	Radioimmunoassay

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710,
Christchurch 8140
Phone:(03) 364 0848
Fax: (03) 364 0818
Toll free: 0800-endolab (3636 522)
E-mail: endolab@cdhb.govt.nz

ANP-NT (1-30), Atrial Natriuretic Peptide-NT (1-30)

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2.2ml plasma in iced water, by arrangement only
Transport	Frozen
Storage	-20°C
Species	human

Test Details:

Frequency:	By arrangement only
Reference Interval:	0.13 – 0.91nmol/L
Standard:	pre pro-ANP (26-55)
Method:	Radioimmunoassay

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710,
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: 0800-endolab (3636 522)
E-mail: endolab@cdhb.govt.nz

Anti-Mullerian hormone (AMH)

Anti-mullerian hormone (AMH) is exclusively produced by gonadal tissue, namely the testicular Sertoli cells and the ovarian granulosa cells. In **males**, the major function of AMH is to induce regression of the Mullerian structures (Fallopian tubes, uterus and upper 1/3 vagina) *in utero*. Circulating AMH levels rise rapidly during the first year of male life, are highest during late infancy and gradually decline until puberty. In **females**, AMH is mainly produced by primordial follicles and small antral follicles up to the 4-6mm stage. AMH has an inhibitory effect on primordial follicle recruitment and on the responsiveness of the growing follicles to FSH.

Synonym:

Mullerian-inhibiting substance (MIS)

Expected concentrations:

In females AMH levels are almost undetectable at birth, with an increase in the first 2-4 years of life; then AMH levels are stable until the age of approximately 30 years after which there is a gradual decline reflecting follicular depletion.

AMH is measured in Endolab using the ImmunoTech Enzyme immunoassay, from Beckman Coulter Ltd. The manufacturers report expected values for healthy women, below 38 years of age, with normal follicular status, at day 3 of the cycle, based on 335 samples, to be 29 pmol/L (14 – 49 pmol/L); median and (10th-90th percentile) range.

Applications:

The measurement of serum AMH has been used in a variety of clinical settings, most of which are currently investigational and not a component of routine clinical care. The most well established indications for AMH measurement are the following:

(1) Evaluation of intersex disorders – the persistent Mullerian duct syndrome is a rare form of male pseudohermaphroditism characterised by the persistence of Mullerian structures in otherwise normal males. Broadly, this genetically transmitted disorder may be due to either a mutation in the gene encoding AMH (type 1) or an inactivating mutation in the AMH receptor (type 2). The measurement of AMH predicts the nature of the underlying genetic defect and may allow targeted mutational analysis of the gene for AMH or the receptor. Moreover, AMH measurement can be used to determine testicular status in prepubertal children with impalpable gonads, thus allowing differentiation of anorchidism from bilateral undescended testes in boys with cryptorchidism.

(2) Marker of granulosa cell tumours – AMH levels are increased in more than 75% of women with granulosa cell tumours (GCT's) and seem to be a superior tumour marker compared to α – inhibin and oestradiol in the follow up of GCT's.

AMH measurement has also been used, mostly on a research basis, in the following settings:

(1) Marker of ovarian reserve in assisted reproduction technology (ART) – as AMH is produced by the growing antral follicles (4-6mm) up to the stage of selection of a dominant follicle, it may serve as a marker of ovarian reserve for women undergoing IVF. Studies have suggested that day 3 AMH levels in an IVF cycle predict the number of oocytes retrievable as well as clinical pregnancy outcome. Other studies suggest that IVF patients who develop ovarian hyperstimulation syndrome have 6-fold higher basal AMH levels than controls. However, the precise role that AMH measurement in the IVF setting awaits further detailed investigation.

(2) Polycystic ovary syndrome – serum AMH levels are markedly elevated in patients with polycystic ovary syndrome (PCOS), a reflection of the multiple small antral follicles in this disorder, prompting the suggestion that measurement of AMH may be useful as a surrogate for ovarian ultrasound in the appropriate clinical context. Other work has suggested that treatment of patients with PCOS with metformin decreases AMH and that daughters of women with PCOS have raised AMH when compared with controls. Precise cut-off values to allow routine clinical use will require further studies.

(3) Marker of ovarian reserve in ageing women – AMH levels are undetectable after the menopause or oophorectomy. AMH levels on day 3 of the menstrual cycle show a progressive decrease with age which precedes the follicular phase rise in FSH, decline in inhibin-B and number of antral follicles, suggesting that AMH is the best marker of ovarian ageing and the menopausal transition.

References:

Clinical Endocrinology 2006;64:603-610.

Journal of Clin Endocrinol Metab 2006;91:3760-3762.

Sample Aliquot Requirements:

Laboratory:	Endolab
Anticoagulant:	Plain, gel separator, li-heparin or EDTA
Volume:	0.5ml serum or plasma, minimum 0.2ml
Transport:	Cold (2-8°C for up to 24 hours)
Storage:	≤-15°C
Species:	Human

Test Details

Frequency:	The test is being introduced in 2008, please enquire.
Reference interval:	AMH levels are age and gender related. For interpretation consult an Endocrine or Fertility Specialist.
Standard:	Human AMH (CHO cell supernatant)
Method:	Immunotech EIA kit

Anti-Pituitary Autoantibodies

This test was previously performed by Dr Patricia Crock's laboratory at the Royal Newcastle Hospital, Newcastle, Australia and tests for antibodies to two pituitary cytosolic proteins.

Reference: Crock, P. Journal of Clinical Endocrinology and Metabolism; 83 (2) 609; (1998).

Clinical Applications:

Pituitary mass lesions, especially if associated with diabetes insipidus, "stalk" abnormality and/or ACTH deficiency. Autoimmune background may be relevant.

Sample Aliquot Requirements:

Volume 5 - 10ml of frozen or freeze-dried serum

Possible availability, enquire before sending samples to:

Dr Patricia Crock
HAPS Central Sendaway
Level 7, McAffrey Wing
Royal Newcastle Hospital
Pacific Street
Newcastle, NSW 2300
Australia
Fax: 61-2-4923 6623

Include a copy of the Australian Quarantine Import Permit with the shipment and waybill (obtainable from the recipient in NSW).

Cost:

Australian price plus freight from Christchurch.

AVP, Arginine Vasopressin, Antidiuretic Hormone, ADH

Note: Prior consultation with Endocrinologist is advisable.

Clinical Applications:

Clinical indications include:

Severe hyponatraemia - especially if "ectopic ADH" syndrome is suspected.

In some difficult cases of hypernatraemia or diabetes insipidus.

Note: Diagnosis of diabetes insipidus is usually possible by careful evaluation of water balance, and sequential changes of urine and plasma osmolality after water restriction, and exogenous vasopressin administration (eg Miller test, page 109).

Patient Preparation

Patients should be non-stressed. AVP increases if patient faints.

All patients warranting AVP assays should have serum osmolality determined (as well as plasma sodium) at the time of blood sampling for AVP - and the result should accompany the request. Urine osmolality is also helpful for interpretation.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	>3ml plasma, minimum >0.5ml. Serum is unacceptable.
Transport	Ambient
Storage	Frozen
Species	All except pigs

Test Details:

Frequency:	Weekly
Reference Interval:	♂ & ♀, no drugs, < 5pmol/L
Standard:	Synthetic AVP
Method:	RIA after extraction

Interpretation

Depends on serum osmolality as well as "volume" status. Severe dehydration, hypotension and/or stress (especially nausea and vomiting) will increase AVP. Low serum osmolality will decrease AVP if volume status is normal. The assay detection limit is approximately 0.2pmol/L. Therefore overnight fluid deprivation may be necessary to aid in interpretation of AVP results.

ProBNP, pro Brain Natriuretic Peptide

Clinical Applications:

ProBNP is a hormone secreted by the overloaded ventricle of the heart. Assay of proBNP in plasma is therefore a useful marker of cardiac decompensation, particularly when symptoms (fatigue, breathlessness) begin to appear. Diagnosis of Heart failure in patients with a background of chronic lung disorder can be difficult and in this setting assay of plasma proBNP has been shown to have diagnostic value.

The proBNP assay is specific for the intact 1-76 molecule.

In subjects with left ventricular dysfunction, plasma concentrations of BNP and N-terminal BNP increase. The concentration of N-terminal proBNP in plasma reflects the degree of left ventricular dysfunction and may therefore be used to aid diagnosis of left ventricular dysfunction and heart failure. Preliminary data suggests that changes in NT-proBNP concentration could be used to evaluate or guide treatment in patients with left ventricular dysfunction or heart failure.

Interpretative advice for proBNP results:

1. Normal range in healthy subjects is <40pmol/L.
2. Values greater than 220pmol/L strongly suggest heart failure in a newly symptomatic (breathless) patient.
3. In between these levels, heart failure is still possible, but all clinical information must be taken into account. ProBNP may be elevated by renal failure, atrial fibrillation, LVH, Valve disease, after myocardial infarction, in the elderly and with severe renal impairment. ProBNP may be decreased by hypothyroidism, treatment with diuretics, vasodilators and ACE-inhibitors.
4. Use of serial measurements to adjust therapy for heart failure (rather than single tests for diagnosis) is experimental. Such repeat measures should generally be no more frequent than 2-3 weeks apart in ambulant outpatients undergoing changes in treatment.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA preferred. Lithium heparin and serum also acceptable
Volume	1ml plasma
	Separate blood within four hours of collection.
Transport	Ambient <3 days, otherwise frozen
Storage	-20°C
Species	human

Test Details:

Frequency:	Research assay only, not for clinical use.
Reference Interval:	<40pmol/L Levels of >220pmol/L strongly suggest heart failure.
Standard:	
Method:	Elecsys 2-site chemiluminescence assay.

BNP - NT, Aminoterminal Brain Natriuretic Peptide, NT-BNP

Synonyms:

N-BNP, Aminoterminal B-type Natriuretic Peptide, Aminoterminal pro-BNP.

Clinical Applications:

NT-BNP (Brain Natriuretic Peptide) is a hormone cosecreted along with BNP by the overloaded ventricle of the heart. Because NT-BNP accumulates within the circulation, blood levels are higher than BNP in established heart failure. Assay of NT-BNP in plasma is therefore a useful marker of cardiac decompensation, particularly when symptoms (fatigue, breathlessness) begin to appear. Diagnosis of Heart failure in patients with a background of chronic lung disorder can be difficult and in this setting assay of plasma NT-BNP has been shown to have diagnostic value.

The sensitivity and specificity (in the recognition of heart failure) are still being evaluated. In addition to heart failure, NT-BNP levels are likely to be raised after acute myocardial infarction, and in some patients with renal failure (plasma creatinine >0.3 mmol/L). Values are expected to fall with effective treatment for congestive heart failure.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2.2ml plasma, minimum 1.1ml plasma Draw blood (5ml) into EDTA tube. Separate off the plasma and freeze within 4 hours of drawing the sample Store the plasma frozen at -20°C.
Transport	Frozen
Storage	-20°C for 3-4 months -80°C for at least 20 months
Species	human

Test Details:

Frequency:	variable. Depends at present on research needs.
Reference Interval:	<50pmol/L Plasma levels indicating ventricular function to follow.
Standard:	NT-BNP (1-21)
Method:	Extracted RIA

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710,
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

BNP - research, Brain Natriuretic Peptide

This assay is available for research purposes.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2.2ml plasma, minimum 1.1ml. Draw blood (5ml) into EDTA tube. Separate off the plasma and freeze within 4 hours of drawing the sample Store the plasma frozen at -20°C.
Transport	Frozen
Storage	-20°C
Species	human

Test Details:

Frequency:	by arrangement only
Reference Interval:	3-12pmol/L
Standard:	BNP (77-108)
Method:	Extracted RIA

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710,
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

Bone alkaline phosphatase

Clinical Applications:

Monitoring disease activity and the response to antiresorptive therapy in patients with Paget's disease.

Monitoring the effectiveness of antiresorptive therapy in patients with osteoporosis.

Determining the degree of bone turnover in patients with various forms of metabolic bone disease (renal osteodystrophy, hyperparathyroidism)

Patient Preparation:

Bone alkaline phosphatase has a long half-life of 24-48 hours and shows minimal circadian variation. Samples may thus be taken at any time of day in the non-fasted state.

Sample aliquot requirements:

Laboratory	Endolab
Anticoagulant	serum, heparin
Volume	0.5ml
Transport	frozen or 4°C up to 48hrs
Storage	frozen or 4°C up to 48hrs
Species	

Test Details

Frequency	weekly
Reference interval	adult males <20.1 µg/L
	pre-menopausal females <14.3 µg/L
	post-menopausal females <22.4 µg/L
Standard	bone alkaline phosphatase (human cell line)
Method	Beckman Coulter Access analyser Ostase assay

Interpretation

Bone ALP is a glycoprotein localised in the plasma membrane of osteoblasts. The precise role is unclear although it is essential for mineralisation. Total circulating ALP is also derived from liver, intestine, spleen, kidney, placenta (in pregnancy) or from various tumours. Bone ALP comprises approximately 50% of total circulating ALP in normal subjects. Measurement of bone ALP by IRMA reflects bone turnover more specifically and sensitively than total ALP. It is however important to note that the specificity for bone ALP is relative as 100 IU/L of liver ALP activity gives a bone ALP result of 2.5 to 5.8 µg/L in the bone ALP assay. Serum samples with significant elevations of liver ALP activity may therefore yield elevated results in this assay.

The minimal significant change of bone ALP is 25%. Potential clinical uses include:

(1) Paget's disease - bone ALP (together with β CTX) is a useful marker of the activity of Paget's disease and the response to anti-resorptive therapy.

(2) Osteoporosis - in patients treated for osteoporosis the percentage change of bone ALP after effective anti-resorptive treatment is typically 50% decline at 3-6 months after initiating biphosphonate therapy. Furthermore, for a group of patients (but not necessarily for the individual patient) there is a close correlation between the reduction in bone turnover assessed at 3-6 months and the ultimate increase in bone density measured by DEXA at 1-2 years. The clinical utility of routinely monitoring the efficacy of antiresorptive therapy in individual patients treated for osteoporosis is not firmly established. However, in patients with particularly severe osteoporosis or apparent resistance to antiresorptive therapy there may be a role for monitoring bone turnover markers (bone ALP and β CTX) after initiating therapy to confirm the efficacy of antiresorptive therapy

C-Peptide - plasma, urine

Clinical Applications:

May be of value in the assessment of residual pancreatic function in insulin treated diabetics, diagnosis of insulinoma and for investigation for covert insulin administration. It is suggested that an endocrinologist be consulted.

Patient Preparation

Note: Fasting and 2 hour postprandial plasma glucose levels are useful INITIAL screening tests. Please send these results with C-peptide.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA but serum and heparin samples also acceptable. Fluoride gives low values.
Volume	Plasma: 0.5ml plasma, minimum 0.3ml Urine: 0.5ml representative of a 24 hr urine collection.
Transport	Ambient for EDTA, heparin and serum for up to 5 days. Urine ambient for up to 24 hours.
Storage	Frozen
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	Plasma: 350 - 750pmol/L (fasting) Urine: 24 hr: 5740-60300pmol/24hr
Standard:	IRR 85/150
Method:	Roche Elecsys

Interpretation

A deficient plasma C-Peptide response to a defined protein/carbohydrate meal is seen in diabetes mellitus. For most clinical purposes the 24-hr urinary C-Peptide excretion gives an adequate and more convenient index of endogenous pancreatic function. Excessive urinary C-Peptide excretion is seen in insulinoma. Deficient C-Peptide excretion is seen in diabetes mellitus and in hypoglycaemia due to the administration of exogenous insulin.

C-Terminal Telopeptide

Clinical Applications

The assay is specific for an octapeptide in the C-terminus of the α_1 chain of type 1 collagen and accurately reflects osteoclast-mediated bone resorption. There is a significant diurnal variation (peak serum CTX between 2-6am) which is blunted after fasting. Samples should thus optimally be collected fasting in the early morning and specimen collection should be consistent during monitoring visits.

Clinical applications include

1. Assessing bone turnover (osteoporosis, hyperparathyroidism, Paget's disease, thyrotoxicosis, immobility)
2. Evaluating the efficacy of anti-resorptive therapy - there is a mean 70% fall of β -CTX from baseline 3-6 months after initiating biphosphonate therapy and 50% after oestrogen replacement. Preliminary data suggests that a decline of >40% in β -CTX at 6 months has a 90-95% positive predictive value that a decline in bone density will be prevented on repeat DEXA scan 2 years later
3. Prospective studies demonstrate that levels of bone resorption markers (including β -CTX) are associated with increased rate of bone loss and fracture risk independently of bone density. However, the translation of these findings into clinical practice requires further study.

Patient preparation

The patient should be fasted overnight 8 hours prior to blood collection in the morning.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	0.5ml EDTA plasma to be taken between 8am and 9am. Note: any repeat sampling on the same patient is to be done at approximately the same time of day
Transport (water)	ambient (if non-haemolysed or haemolysis <1g/L Hb, otherwise frozen or in iced water)
Storage	frozen
Species	human

Test Details:

Frequency:	weekly
Reference Interval:	<0.75 μ g/L (\uparrow 0800-0900hr, \downarrow 1200hr, \uparrow 1500-1600hr)
Standard:	EKAHD- β -GGR
Method:	Roche Elecsys

Interpretation

Increased levels of β -CTx occur in osteoporosis, Paget's disease, primary hyperparathyroidism, renal insufficiency and bone metastases.

Due to the diurnal rhythm of β -CTx it is recommended that samples be taken fasting morning (0800-1000am)

Calcitonin (CT)

Samples can be sent to the Steroid Lab at Christchurch Hospital and these will be sent on a weekly schedule to the Hormone Laboratory at Auckland Hospital. To our knowledge this is the only laboratory in NZ measuring this hormone.

Request must indicate if previously a high value has been obtained and ask "to measure in dilution" (if dilutions are later found to be necessary, they will be charged as extra samples).

For further information contact:

Lablink, phone: (09) 307 8995

Sample Aliquot Requirements:

Laboratory	Hormone Lab, Endocrine Unit, Auckland
Anticoagulant	Serum
Volume	At least 0.6ml serum in pre-chilled tube. Allow to clot 1-3 hr at 4°C then centrifuge at 4°C and freeze immediately. Samples must arrive in the lab in iced water if not frozen. Fasting sample preferred but not essential. Lipaemic samples cannot be used.
Transport	Frozen
Storage	Frozen
Species	Human

Test Details:

Frequency:	Monthly
Reference Interval:	<10 pg/ml
Significant change:	10% at 8pg/ml 3% at 68 pg/ml 2% at 1300 pg/ml

Standard:

Method: IRMA

Diagnostic use:

Tumour marker for medullary thyroid carcinoma (MTC), sporadic or familial, pre- and post-op. Because of the expense of the assay, and the infrequency of medullary thyroid cancer in patients with solitary or dominant thyroid nodules, it is not yet recommended as a screening test in such patients. Because of the early lymph node spread of MTC, calcitonin is commonly elevated after thyroidectomy for MTC, as is carcinoembryonic antigen. Basal hypercalcitoninaemia can occur in patients without medullary thyroid tumours. An unpublished European review of 1200 subjects shows that mild basal calcitonin elevation to >10 pg/ml can occur in some situations:

Chronic renal failure	<100 pg/ml
Hyperparathyroidism	<25 pg/ml
Paget's disease	<25 pg/ml
Other tumours (eg. lung, pancreas)	<250 pg/ml

Is medullary thyroid carcinoma sporadic or familial?

Measurement of calcitonin during a pentagastrin test in family members of patients with sporadic MTC is now obsolete. The presence of RET proto-oncogene germ line mutations that predispose to this tumour can be detected in peripheral blood by the A+ Molecular Genetics Department, Wallace Block (contact via Lablink, 0800-275 875). Thus peripheral blood DNA should be analysed in the patient with MTC. If the family history is negative and the patient presenting with apparently sporadic MTC does not have an identifiable mutation in peripheral blood DNA, then there is little or no likelihood (<1:400) that it is familial and first degree family members do not need to be screened themselves. Confidence is further increased if one of the known mutations is identified in frozen tumour tissue (somatic) but not in peripheral blood (germ line)

Calcitonin response to pentagastrin

Healthy normals	<3 fold increase
Non-MCT hypercalcitoninaemia	<3 fold increase
Medullary thyroid carcinoma	>3 fold increase

Carnitine - plasma, urine, tissue

Clinical Applications:

Carnitine is important in the beta oxidation of fatty acids by acting as an essential co-factor in the transfer of long chain fatty acids across the inner mitochondrial membrane. The acyl groups are esterified to the hydroxyl group on carbon 3 of the molecule.

Carnitine deficiency is most commonly investigated in infants. A number of inherited metabolic defects are known which result in accumulation of neutral lipid within the skeletal muscle, myocardium and liver, resulting in muscle weakness and lipid storage myopathy. Secondary carnitine deficiency is associated with other inborn errors of metabolism in which abnormally high levels of acyl-CoA react with carnitine resulting in a relative insufficiency.

Sample Aliquot Requirements:

Laboratory	Paediatric laboratory section of biochemistry
Anticoagulant	Plain, heparin, EDTA
Volume	Plasma or serum: >0.5ml Urine: 0.5ml Tissue: 0.1g minimum
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	monthly
Reference Interval:	plasma: adults: 23-60 $\mu\text{mol/L}$ urine: total: 30-100 mmol/mol creatinine free: 20-50 mmol/mol creatinine tissue: dependent on tissue type and clinical background
Standard:	
Method:	HPLC

For further details contact:

Canterbury Health Laboratories
P O Box 151
Christchurch 8140
Phone: (03) 364 0332
Fax: (03) 364 0750

Catecholamines - plasma

This assay reports plasma Adrenaline and Noradrenaline (Epinephrine and Norepinephrine) levels. Dopamine is reported only if specifically requested.

Clinical Applications:

The measurement of plasma catecholamines lacks both sensitivity (84%) and specificity (81%) for the diagnosis of Pheochromocytoma and Endolab is thus no longer offering plasma catecholamines as a routine clinical test. Accumulating evidence suggests that measurements of plasma free metanephrines or urinary fractionated metanephrines (normetanephrine and metanephrines separately) are the most sensitive tests for the diagnosis of Pheochromocytoma. Plasma metanephrines have a high sensitivity (99%), but lack specificity (89%). Therefore the suggested approach to diagnosis is to screen with 24 hour urinary fractionated metanephrines and catecholamines. Plasma metanephrines are useful in equivocal cases and particularly in syndromic forms of phaeo (MEN2 and Von Hippel Lindau) where elevation in plasma metanephrines may precede any other biochemical abnormality. In future, to limit false positive and negative results, clinical samples sent to Endolab with requests for catecholamines as a screen for phaeo will be assayed for plasma metanephrines. If there is a strong clinical indication for the measurement of plasma catecholamines, please contact Endolab directly and assay can be arranged.

Patient Preparation

Since many factors alter plasma catecholamine levels, patient preparation is important.

Avoid smoking and caffeine intake 12 hours before blood sampling. Ideally the patient should fast overnight prior to venous sampling in the morning.

Insert a venous line (butterfly is satisfactory), and flush with a heparin-saline solution (10 U/ml), and occlude. When the line for blood drawing is secured, the patient is left recumbent in non-stimulating surroundings for 30 minutes.

At 30 minutes, clear the venous line then withdraw the sample (Supine) of 5ml blood for catecholamine measurement.

Note: Blood samples taken during or soon after a hypertensive crisis (or similar suspicious event) may be helpful for diagnosis of phaeochromocytoma.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2.1ml plasma preferred; however absolute minimum volumes for a non-repeatable result are: Adrenaline & Noradrenaline = 1.1ml Noradrenaline only = 0.2ml Take blood into a 5ml vacutainer tube containing EDTA anticoagulant, which should be chilled in crushed ice & water prior to blood sampling. Invert the tube several times to mix blood with anticoagulant. DO NOT SHAKE. Immediately place the tube in an ice & water bath. Centrifuge at +4°C within 15 minutes of collection. Aspirate the plasma taking care to avoid the buffy coat of cells at the interface (platelets contain high levels of catecholamines). Store and transport the plasma frozen (ideally at -80°C).
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	Adrenaline <570pmol/L Noradrenaline 470-3800pmol/L Dopamine (when requested) <510pmol/L
Standard:	
Method:	HPLC-EC

Interpretation

Many medications alter circulating catecholamines therefore PLEASE NOTE DRUG THERAPY on the request form to assist interpretation of the result.

Most phaeochromocytoma cases will exhibit plasma catecholamine levels at least several-fold normal. Borderline or modest elevation can reflect anxiety or other stimuli, including volume depletion, sepsis, post-trauma response etc. Modest elevations should be interpreted with great caution and if the clinical picture remains suggestive repeat sampling plus or minus a **clonidine suppression test** should be considered.

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710,
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

For clinical interpretation, please contact

Dr Steven Soule
Department of Endocrinology
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone (03) 364 0927
Fax: (03) 364 1159

Catecholamines - urine

Analytes include Noradrenaline, Adrenaline and Dopamine.

Clinical Applications:

Catecholamines are quantitated to screen for two tumours, which secrete elevated levels. These are neuroblastoma and phaeochromocytoma.

This procedure quantitates dopamine, adrenaline and noradrenaline. Normetadrenaline and metadrenaline can also be done on the same sample by request. All values are reported as an age related creatinine ratio.

Patient Preparation

In patients under 15 years of age, VMA may be assayed on the same sample. Patient should avoid all vanilla containing foods prior to collection. In older patients, VMA is not helpful.

Please note any drugs that the patient may be taking as a number interfere with the catecholamine & VMA assay.

Sample Aliquot Requirements:

Laboratory	Specialist Lab
Anticoagulant	
Volume	20ml aliquot (a urine pottle full) of a 24 hr urine which has been collected over acid. Acidified bottles available from Canterbury Health Laboratories. State collection time(s) and total volume Random samples may also be sent for children only where a 24hr collection is not possible. A full urine pottle is required if an aliquot of a 24 hr sample is sent.
Transport	Keep refrigerated
Storage	Keep refrigerated
Species	

Test Details:

Frequency:	2x weekly (Monday + Thursday)
Reference Range:	Adult: Adrenaline: <36 $\mu\text{mol/mol}$ creatinine Noradrenaline: <70 $\mu\text{mol/mol}$ creatinine Dopamine <332 $\mu\text{mol/mol}$ creatinine Child: age dependant.
Standard:	
Method:	Resin extraction, HPLC

Interpretation

VMA is a metabolite of adrenaline & noradrenaline and as such is a useful screening procedure for investigation of catecholamine disorders.

We now only quantitate VMA in young children or in cases of suspected or confirmed neuroblastoma.

Cortisol - serum, plasma

Clinical Applications:

In diagnosis of Cushing's syndrome.

In diagnosis of adrenal hypofunction (including Addison's disease, hypopituitarism, congenital adrenal hyperplasia and drug induced adrenal atrophy). If considering **primary** adrenal insufficiency also measure plasma ACTH.

Sometimes useful as a check on attained blood levels in patients taking maintenance cortisol or cortisone therapy.

Patient Preparation

Avoid non-essential drugs and non-specific stress where possible. In hypofunction states, sampling is best undertaken at 08.00 hr or early morning hours whereas in Cushing's Syndrome more is gained by sampling when levels are normally low (2400 hr).

Patient need not be fasting but should not be stressed. Time of sampling must be stated.

Glucocorticoid preparations (other than dexamethasone) should be discontinued if possible at least 24 hours previously.

In patients presenting acutely unwell with shock or hyponatraemia etc. and where cortisol deficiency is suspected, draw blood at first presentation for cortisol (and ACTH). Note clinical status and time of sampling.

Additional tests

Overnight dexamethasone testing may be valuable in the diagnosis of Cushing's Syndrome. In this test 1.0 mg dexamethasone is taken orally at 2400 hr and plasma cortisol is drawn at 08.00 hr the following morning (see page 99).

Synacthen test (response to 0.25 mg synacthen IM) is invaluable in the diagnosis of adrenal insufficiency (see page 113).

Blood is drawn for cortisol before and 30 minutes after soluble synacthen given IM.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	
Volume	0.1ml serum or plasma, minimum 0.05ml
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	Daily
Reference Interval:	08.00: 250 - 800nmol/L 10.00: 110 - 570nmol/L 16.00: 200 - 330nmol/L 24.00: < 300nmol/L
Method:	ELISA

Interpretation

Cortisol

Raised values: stress, Cushing's syndrome, patients taking oestrogenic hormones.

Low values: Adrenal insufficiency, adrenal enzyme defect, adrenalectomy, and long term glucocorticoid therapy. Use plasma ACTH levels to interpret low cortisol levels if Addison's disease is suspected.

For suspected Cushing's syndrome, a 1mg overnight dexamethasone suppression test (1mg dex at midnight, plasma cortisol at 0800h) and/or 24h urine free cortisol (and creatinine) is recommended.

In the evaluation of suspected adrenal insufficiency, 0800-0900 cortisol <100nmol/L is abnormal and requires endocrine review.

In the setting of critical illness, the criteria for hypoadrenalism remain uncertain. A random cortisol between 550 and 950nmol/L does not necessarily exclude hypoadrenalism.

For suspected adrenal insufficiency and intermediate cortisol values (100-550nmol/L), a synacthen test is recommended.

Synacthen Test.

Low values: Addison's disease, hypopituitarism, long term glucocorticoid therapy etc.

Normal Synacthen response: increase to a level of 550nmol/L or greater. Post-synacthen cortisol of >550nmol/L is usually normal, although if clinical suspicion remains high, discussion with an Endocrinologist is recommended.

Post-synacthen cortisol of <550nmol/L is abnormal. Endocrine review is recommended.

Overnight dexamethasone test:

Failure of cortisol to fall after 1 mg dexamethasone at midnight is seen in Cushing's syndrome, patients with stress (eg marked depression, fever etc) and in patients taking phenytoin or other hepatic microsomal inducer drugs.

Sample taken post 1mg Dexamethasone.

Cortisol <50nmol/L: Excludes Cushing's syndrome.

Cortisol 50-150nmol/L: Possible Cushing's syndrome. Consider 24 hour urine cortisol followed by discussion with endocrinologist.

Cortisol >150nmol/L: Cushing's syndrome likely. Discuss with Endocrinologist.

Cortisol - Salivary

Salivary cortisol is also offered. This test has applications for non-invasive testing, particularly in paediatric and low stress situations.

Sample requirements:

1.0ml saliva in a sterile container.

Interpretation

The salivary cortisol assay is primarily useful as a screening test for Cushing's syndrome. A normal bedtime level should be $<15\text{nmol/L}$. It has also been applied to studies of normal diurnal variation and as a means of assessing adequacy of cortisol replacement, Typically samples are collected at 0800, 1200, 1700 and 2200.

For details of availability and specimens required, please contact:

Dr John Lewis

Steroid Biochemistry

Canterbury Health Laboratories

Phone: (03) 364 0888

Fax: (03) 364 0889

Cortisol - urine

Clinical Applications:

Diagnosis of hypercortisolism. This is a screening test for Cushing's syndrome.

Patient Preparation

No special preparation is required. It is preferable that non-essential drugs be stopped 1 - 2 days prior and during the collection period. The patient should not be receiving corticosteroids.

Additional tests

Dexamethasone suppression may sometimes be employed.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	
Volume	5ml of non-acidified 24-hr urine collection, containing no preservative should be stored deep frozen. Total volume of 24-hr collection should be clearly marked both on the sample and the accompanying Endolab or CHLabs form.
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	2x weekly
Reference Interval:	100 - 400 nmol/24 hr
Standard:	
Method:	ELISA following extraction

Interpretation

High values are seen in Cushing's Syndrome irrespective of cause.

Low values may occur in adrenal insufficiency.

Note: Non-specific stress (fever, surgical operation, recent myocardial infarction etc) endogenous depression and alcoholism may increase cortisol excretion. Impaired renal function will lower excretion rates.

CRH, Corticotrophin Releasing Hormone

Clinical Applications:

A research assay, which may be used for the differential diagnosis of Cushing's syndrome when the possibility of ectopic CRH secretion by a tumour is being considered. Assay subject to delays.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	5ml plasma, minimum 2.5ml plasma
Transport	Frozen or must remain cold
Storage	$\leq 15^{\circ}\text{C}$
Species	Human, rat, horse, sheep

Test Details:

Frequency:	Contact the laboratory.
Reference Interval:	<5pmol/L (higher in late pregnancy)
Standard:	h/r CRH ₍₁₋₄₁₎ Peninsula
Method:	RIA after plasma extraction

For details of availability, please contact:

Dr Jane Ellis
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

DHEA, Dehydroepiandrosterone

DHEA-S Dehydroepiandrosterone-sulphate is the preferred marker to DHEA. The excretion rate of DHEAS is several hundred times in excess of its unsulphated form DHEA. The half-life of DHEA-S is 8-11 hours, compared with 30-60 minutes for DHEA.

DHEA Sulphate and Androsterone/Epiandrosterone Sulphates

Clinical Applications:

Hirsutism, virilisation, 5 α reductase marker.

Patient Preparation

No special preparation. A preference for sampling between 0800-1000 hr exists, since better comparisons with normal subjects are then available. Please state the time of sampling, nature of drug therapy and provide a brief summary of clinical particulars.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, plain
Volume	0.5ml serum or EDTA plasma, minimum 0.2ml
Transport	Ambient
Storage	Cold
Species	

Test Details:

Frequency:	Twice weekly
Reference Interval:	DHEAS ♂ 0.5-12 μ mol/L
	♀ 2.0-12 μ mol/L
	AoS/epiAoS ♂ and ♀: 0.8-6.0 μ mol/L
Standard:	
Method:	ELISA

Interpretation

Dehydroepiandrosterone is secreted by the adrenal, the ovary and the testis and can be converted peripherally to the active androgen, testosterone. Clinically, because of their association with Testosterone, DHEA and Androstenedione are involved in the production and maintenance of male secondary sex characteristics. Elevated levels occur in patients with androgen-producing tumours and in enzyme deficient states which effect an increase in androgen production.

The normal range for androsterone/epiandrosterone sulphate for both males and females is 0.8-6.0 μ mol/L. Subnormal levels are evident in 5 α reductase deficiency and dramatic reductions compared to basal levels are seen following administration of 5 α reductase inhibitors.

Note: The DHEAS ELISA is run using tandem monoclonal antibodies, allowing the assay of DHEAS and androsterone/epiandrosterone sulphates. The latter two sulphates comprise the major 5 α reduced steroids in plasma, hence an index of 5 α reduced activity is also possible.

Dihydrotestosterone (DHT)

Clinical Applications:

It should be used primarily as a marker of testosterone 5-alpha-reductase activity, with application to hirsutism, virilisation gonadal dysgenesis, androgen insensitivity and cancer of the prostate.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, heparin, plain
Volume	0.5ml EDTA plasma, take at 0900 hr, minimum 0.2ml
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	Available only by prior consultation
Reference Interval:	♂ 1000-6000pmol/L ♀ 50-600pmol/L
Standard:	
Method:	RIA kit

Interpretation

The test involves the measurement of both total testosterone (T plus DHT) and DHT levels. The ratio of these two values when referred to normal ranges for men and women provide an index of 5-alpha-reductase activity.

For further details contact:

Dr P Elder or Dr J Lewis
Steroid Laboratory
Canterbury Health Laboratories
PO Box 151
Christchurch 8140
Phone: (03) 364 0888
Fax: (03) 364 0889

DNA Sample Addresses

Suspected Men 1:

Contact:

Cytogenetics
Canterbury Health Laboratories
Christchurch
New Zealand

20ml heparinised blood will be taken for preparation of an EBV line.

The sample will be sent to:

Dr Nick Heyward
Queensland Institute of Medical Research
Brisbane
Australia
Phone: 61-7-362 0306
Fax: 61-7-362 0107

Suspected Men 2:

Dr Robinson must be contacted first, as an import permit will be required.

Dr Bruce Robinson
Royal North Shore Hospital
Sydney
Australia
Phone: 61-2-438 7267
Fax: 61-2-439 2798

Endothelin

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	3.2ml plasma. Collect in iced water, separate and freeze as soon as possible
Transport	Frozen
Storage	Frozen @ $\leq -15^{\circ}\text{C}$
Species	human, sheep

Test Details:

Frequency:	A research assay only, frequency depends on research requirements.	
Reference Interval:	Human:	0.9 - 2.3pmol/L
	Sheep:	1.1 – 3.2pmol/L
Standard:		
Method:	RIA	

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

Erythropoietin

Sample Aliquot Requirements:

Laboratory	Endocrine laboratory, Waikato Hospital
Anticoagulant	plain
Volume	2ml plain
Transport	Frozen
Storage	
Species	

For further details contact:

Sue Carnoutsos,
Special Tests Section of Haematology
Canterbury Health Laboratories
P O Box 151
Christchurch 8140
Phone: (03) 364 0375
Fax: (03) 364 0750

FSH, Follicle stimulating hormone

Clinical Applications:

Investigation of hypogonadism or delayed puberty.

1. Investigation of oligospermia or azospermia (a raised FSH value usually indicates that the defect in spermatogenesis is gross).
2. Investigation of primary and secondary amenorrhoea.
3. Assessment of hypothalamic-pituitary function. Low serum FSH values cannot always be distinguished readily, (depending on the sensitivity of the assay), from normal values except in postmenopausal women who have higher resting values than pre-menopausal. A premenopausal value in postmenopausal women is suggestive of hypothalamic-pituitary disease.

Note: Serum FSH determinations generally provide a better indication of primary gonadal failure than serum LH.

During the menopausal transition, which may last several months or longer, FSH levels may fluctuate considerably.

Patient Preparation

Patient - Need not be fasting. Time of sampling is not critical. Details of age, gender and last menstrual period are essential, and also whether the patient is taking oral contraceptives or sex steroids.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	Serum or lith. heparin plasma (EDTA plasma acceptable but results may be 5% lower)
Volume	0.5ml serum or plasma, minimum 0.2ml
Transport	Ambient
Storage	≤-15°C
Species	Human

Test Details:

Frequency:	daily (Monday - Friday)		
Reference Interval:	0-9yrs	0-6.5 IU/L	
	approx 10-15yrs	“Prepubertal range 0-6.5IU/L. Levels rise during puberty towards adult range”.	
	Adult male	2-12 IU/L	
	Adult female	Follicular	3-10 IU/L
		Mid Cycle	4-25 IU/L
		Luteal	2-8 IU/L
		Post Menopause	>20 IU/L
	Antenatal	<1 IU/L	
Standard:	2nd IRP (78/549)		
Method:	Access Analyser, Beckman/Coulter		

Interpretation

1. Raised values: after menopause, midcycle peak (less affected than LH), primary gonadal failure (eg Klinefelter's syndrome, Turner's syndrome, gonadal dysgenesis or agenesis, bilateral torsion of the testis, orchidopexy, testicular injury, premature ovarian failure, oligo or azospermia).
2. Low values - ie repeatedly low or no response to stimulation. Hypothalamic - pituitary disease (eg pituitary ablation, pituitary tumours, craniopharyngioma, Kallmann's syndrome etc.)
3. Serum FSH determinations generally provide a better indication of primary gonadal failure than serum LH.
4. During the menopausal transition, which may last several months or longer, FSH levels may fluctuate considerably.
5. In males, FSH tends to increase with age. Levels of 2-8 IU/L are expected for adults <40 years and 2-14 IU/L for >40 years.

Gastrin

Clinical Applications:

To aid in the diagnosis of the Zollinger-Ellison syndrome

Patient Preparation

Patient should be fasting overnight.

Sample Aliquot Requirements:

Laboratory	Specialist Lab
Anticoagulant	Plain
Volume	1ml serum. Blood should be placed in iced water if more than 1 hour will elapse between time of sampling and separation of the serum
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	<150 ng/L
Standard:	Synthetic G17
Method:	RIA

Interpretation

In the Zollinger-Ellison syndrome serum gastrin levels are elevated sometimes to very high levels. However, gastrin levels may also be elevated in other situations and interpretation must be cautious. A knowledge of whether or not the stomach secretes acid helps to interpret the serum gastrin data.

Hyperacidity: Zollinger-Ellison Syndrome (Gastrinoma)
Antral hyperplasia
Chronic renal failure
Duodenal ulcer (modest elevation occasionally)

Hypoacidity: Gastric ulcer
Truncal vagotomy

Achlorhydria: Type A Gastritis (as found in pernicious anaemia)

Elevated gastrin levels are often found in pernicious anaemia in association with positive parietal cell antibodies.

When in doubt, a secretin test may help. GIH Secretin 2 units/kg IV as a bolus injection normally causes the serum level to fall. In the Zollinger-Ellison Syndrome serum gastrin paradoxically rises. For this test collect serum at 0, 15, 30 and 45 minute intervals.

GhRelin - research

This assay is available for research purposes.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	1ml plasma, minimum 0.5ml
	Draw blood (5ml) into EDTA tube.
	Separate off the plasma and freeze within 4 hours of drawing the sample.
	Store the plasma frozen at -20°C
Transport	Frozen
Storage	-20°C
Species	Human

Test Details

Frequency	By arrangement only
Reference interval	Not yet established
Standard	GhRelin (1-28)
Method	Extracted RIA

For details of availability and specimens required, please contact:

Dr Chris Pemberton
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone (03) 364 0848
Fax (03) 364 0818
Toll free: 0800-endolab (0800-36 36 522)
E-mail: endolab@cdhb.govt.nz

Glucagon

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA plasma is preferred, Serum and Heparin plasma are acceptable.
Volume	1ml plasma
Transport	EDTA samples: 4°C for up to 5 days or frozen
Storage	Frozen
Species	pig and cattle glucagon can also be measured

For details of availability, please contact:

Dr J Livesey
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

Glucocorticoid Remediable Aldosteronism (Familial Dexamethasone Suppressible Hyperaldosteronism) Gene Testing

A genetic screening test is now available in Christchurch for identifying subjects with the hybrid aldosterone synthase gene associated with the dexamethasone suppressible form of hyperaldosteronism. Such subjects are likely to present with low renin hypertension - or mimic some of the features of primary aldosteronism. However, familial studies indicate that none of these features need be present though abnormal aldosterone/renin ratios are present in most cases.

Subjects worth screening:

Any person with high plasma aldosterone/renin ratio or suspicion of primary hyperaldosteronism (especially if no adenoma found). Also where close relatives have a similar form of hypertension.

A case can be made for screening all new hypertensives (say under the age of 50 years) in order to determine gene frequency in Christchurch.

Procedure:

Take 10ml of EDTA blood and request DNA screen for Familial Hyperaldosteronism. Blood should not be centrifuged and should be sent to:

Molecular Pathology,
Christchurch Hospital,

Or

Dr Peter George, Canterbury Health Labs.

GH, Growth Hormone, hGH, Somatotropin

Clinical Applications:

Diagnosis of Acromegaly and Gigantism. Note that a single raised value is not diagnostic as growth hormone levels fluctuate considerably in normal individuals. IGF-1 is a better screening test. For the diagnosis of acromegaly a glucose suppression test (see page 106) or repeated sampling are necessary. Mean growth hormone levels of $<2.5\mu\text{g/L}$ indicate biochemical remission of acromegaly. The mean level should be calculated on a minimum of 5 samples at 30 - 60 minute intervals. Growth hormone determinations are also useful in assessing the response to pituitary surgery, irradiation, or medical therapy.

Differential Diagnosis of Short Stature. At least two formal stimulatory tests of growth hormone secretion (eg insulin hypoglycaemia (page 106), clonidine (page 102), arginine infusion (page 102) glucagon (page 57) are necessary before a diagnosis of growth hormone deficiency can be made. Sex steroid priming may help to clarify the interpretation of results in pre-pubertal children with a borderline growth hormone response. A plasma GH level 10 minutes after a 10 minute period of vigorous exercise is another useful screening test. If raised, further formal stimulatory tests can be avoided.

Assessment of Hypothalamic Pituitary Function. Stimulation tests of growth hormone secretion (see above) are necessary to demonstrate growth hormone deficiency.

Patient Preparation

Patient should be unstressed and preferably fasted prior to sampling. Repeated sampling, stimulation and suppression tests are usually necessary when investigating disorders of growth hormone secretion (see above). It is essential to state the age of the patient and percentage above ideal body weight in order to facilitate interpretation of the results.

Note: Hypothyroidism must be corrected before carrying out tests of growth hormone secretion. Other drugs, particularly progestogens, chlorpromazine, L-Dopa, etc should be avoided.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA, heparin
Volume	0.5ml plasma or serum. minimum 250 μl
Transport	Ambient overnight or at 4°C within 5 days.
Storage	Frozen
Species	Human
Identification	All samples, including those in a series, must have individual unique lab ID numbers.

Test Details:

Frequency:	Weekly
Reference Interval:	♂ (adult): $<1.0\mu\text{g/L}$ ♀ (adult): $<10\mu\text{g/L}$
Standard:	human GH
Method:	IRMA

Interpretation

Raised Values - exercise, stress (physical and psychological), sleep (stage IV), protein foods, arginine, hypoglycaemia, glucagon, L-Dopa, acromegaly, gigantism, some diabetics.

Low Values - (ie impaired response to stimulation tests) hypothyroidism, hypopituitarism, obesity, prepubertal status, bromocriptine, L-Dopa, somatostatin, glucose infusion.

GH levels of $>1\mu\text{g/L}$ 2 hours after ingestion of 75g oral glucose is diagnostic of acromegaly.

cGMP, Cyclic Guanosine Monophosphate

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA, serum unacceptable
Volume	1.1ml plasma
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	By prior arrangement only
Reference Interval:	
Standard:	
Method:	RIA after extraction

For details of availability and specimens required, please contact:

Dr T G Yandle
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: (0800-endolab (3636 522))
E-mail: endolab@cdhb.govt.nz

hCG, Human Chorionic Gonadotrophin (β -subunit) – serum

Clinical Applications:

Detection and management of gestational trophoblastic disease (eg molar pregnancy, choriocarcinoma).

Diagnosis and management of trophoblastic tumours in both males and females.

Patient Preparation

No special preparation. Please state time of sampling and, when applicable, nature of drug therapy, time from evacuation of mole or removal of tumour. Please provide brief summary of clinical particulars.

Note: Urinary HCG haemagglutination tests provide a rapid screen when elevated HCG levels are suspected.

Sample Aliquot Requirements:

Laboratory	Specialist Lab
Anticoagulant	Plain preferred
Volume	0.5ml serum
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	2-3x weekly
Reference Interval:	non-pregnant adults 0-5 IU/L
Standard:	2 nd IS
Method:	AxSYM

Interpretation

In the follow-up of treated patients serum concentrations of HCG may take several weeks to decline to non-detectable levels after the primary treatment. Continuing measurable levels or the re-appearance of HCG indicate the presence of active trophoblastic tissue.

For urgent tests and/or further details contact:

Canterbury Health Laboratories
P O Box 151
Christchurch 8140
Phone: (03) 364 0332
Fax: (03) 364 075025

IGF-1, Somatomedin, Insulin-like Growth Factor-1

Clinical Applications:

Acromegaly: IGF-1 levels are elevated in active acromegaly and provide a good guide to activity of the disorder after treatment. Diagnosis of acromegaly also requires demonstration of autonomous growth hormone secretion (see Growth Hormone, page 59).

Growth hormone deficiency: IGF-1 measurements are a relatively insensitive test for growth hormone deficiency. Levels are low in normal children aged < 3 years, children with Laron dwarfism and some patients with classical growth hormone deficiency. IGF-1 levels may also be reduced in panhypopituitarism, hypothyroidism and malnutrition.

Sample Aliquot Requirements:

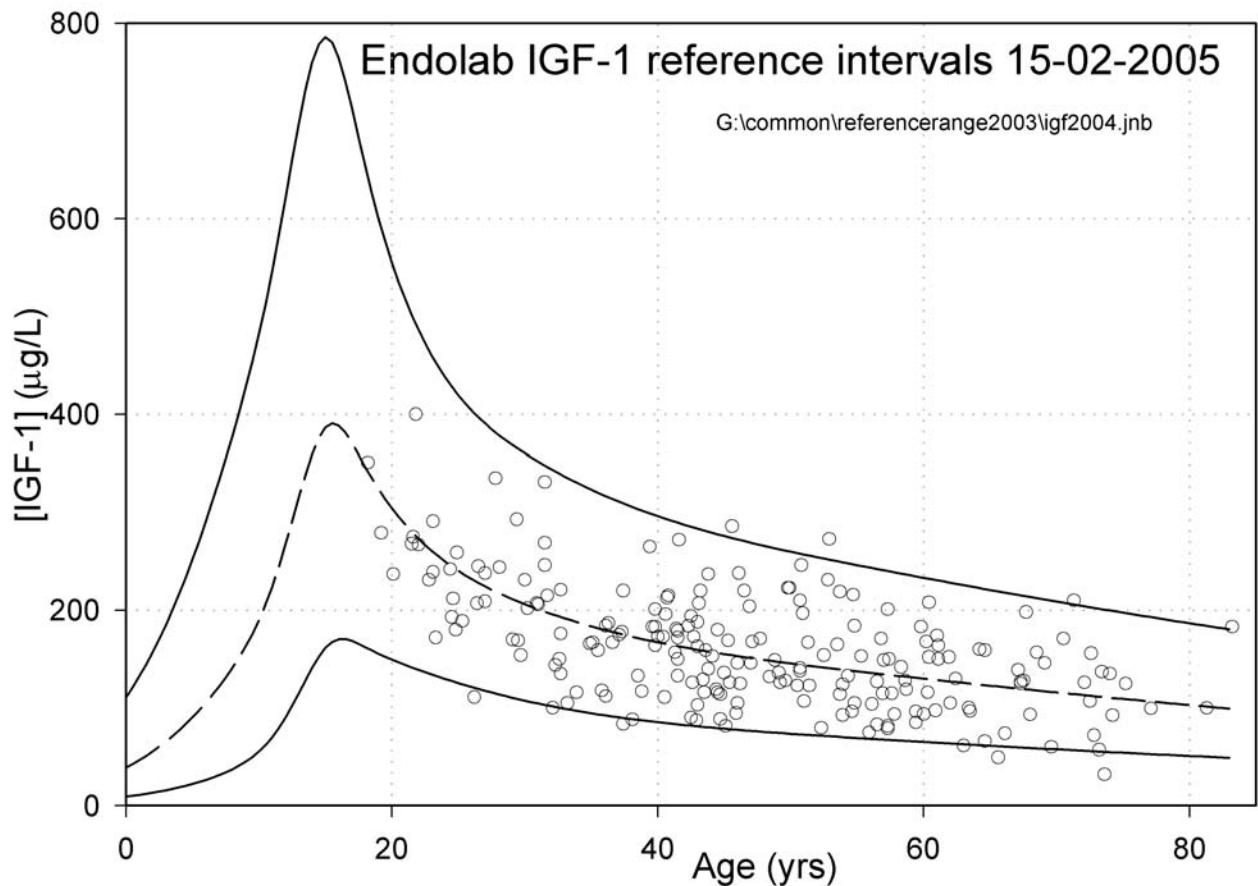
Laboratory	Endolab
Anticoagulant	EDTA serum, heparin
Volume	0.5ml plasma or serum, minimum 0.15ml plasma or serum
Transport	Ambient
Storage	Frozen
Species	Human, equine, bovine, porcine, canine, guinea-pig

Test Details:

Frequency:	Weekly
Reference Interval:	See graph
Standard:	Recombinant IGF-1
Method:	RIA after extraction

Interpretation

See above notes for effects of young age, puberty, etc.



IGF-BP3 Insulin-like Binding Protein-3

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA, serum
Volume	0.1ml plasma or serum, minimum 0.05ml plasma or serum
Transport	Frozen preferred, >3 days liquid rejected
Storage	Frozen
Species	

Test Details:

Frequency:	Monthly
Reference Interval:	
Standard:	
Method:	Bioclone RIA

Inhibin-B

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	serum
Volume	1ml serum
Transport	Frozen
Storage	Frozen
Species	Human

Test Details:

Frequency:	3-6 monthly	
Reference Interval:	Normal post-menopausal women	<30pg/ml
	Normal menstrual cycle (not on combined OC)	<250pg/ml
	Normal men	100-200pg/ml
Standard:		
Method:	DSL Inhibin-B kit	

For further information:

Dr Margaret Evans
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0886
Fax: (03) 364 0818
Freephone: 0800 endolab (36 36 522)
E-mail: endolab@cdhb.govt.nz

Insulin and Insulin Antibodies

Clinical Applications:

Insulinoma – The concentration of insulin is disproportionately high in relation to the concurrent low, (<2.5mmol/L) fasting plasma glucose level. See Turner et al.¹ Ideally insulin levels are measured during an hypoglycaemic episode and/or after a carefully supervised 48-72 hour fast.

The secretion of ProInsulin and C-Peptide may also be increased in patients with insulinoma. These assays can be carried out by special arrangement with Endolab.

Tests of insulin suppressability may be undertaken using infusion of insulin and measurement of C-Peptide levels when hypoglycaemic.

Hepatic vein insulin levels can be measured to help localise site of pancreatic islet cell adenoma after selective injections of calcium via branches of the celiac axis artery.

Early diagnosis of diabetes – the first phase of insulin release which occurs 3 to 5 minutes after the intravenous injection of 25g glucose (see page 103), is absent or blunted. Subsequent insulin secretion may be normal or increased. A raised fasting insulin value or an excessive response to glucose may suggest insulin resistance such as occurs in obesity, acromegaly, Cushing's Syndrome, etc.

Note: For clinical purposes the conventional 75g oral glucose tolerance test or 25g IV glucose tolerance test gives adequate information and plasma insulin determinations are generally unnecessary.

Patient Preparation

Simultaneous glucose levels are needed to interpret results.

Patient must be fasted for intravenous glucose tolerance test and have an adequate carbohydrate intake 3 days previously (see page 107). Patients with suspected insulinoma should be fasted for 15 – 18 hours prior to sampling for plasma insulin and glucose, provided blood sugar values allow this. If possible, blood should be drawn for insulin and C-peptide as well as glucose when spontaneous hypoglycaemia occurs – since such measurements can be diagnostic in insulinoma.

Note:

The results of concurrent plasma blood glucose determinations must be sent with the samples for insulin assay, as isolated plasma insulin values are meaningless.

Biguanides, sulphonylurease and β blocking drugs should be discontinued prior to testing.

Does not measure Lispro insulin.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA preferred (most stable) Heparin and serum are acceptable Also fluoride tube for glucose assay, if testing for insulinoma
Volume	0.5ml plasma or serum for insulin, minimum 0.2ml. Centrifuge promptly, then separate off and freeze plasma/serum. 1ml plasma or serum for insulin antibodies.
Transport	Frozen plasma or whole blood in iced water (<4°C)
Storage	Frozen plasma
Species	Human, dog, probably most other species. Lowered cross-reaction with non-human insulins. In dogs only useful when elevated insulin levels are suspected.
Note	Haemolysis must stringently be avoided. The interference increases with temperature and time. At a level of 1.3g Hb/L, insulin is immediately lowered by 10%. At 0.4g Hb/L, insulin is lowered by 10% after 1 hour at room temperature.

Test Details:

Frequency:	daily
Reference Interval:	10-80pmol/L (overnight fast, adult, BMI <25)
Standard:	WHO insulin 1 st IRP Conversion factor: $\mu\text{U/ml} = 0.144 \times \text{pmol/L}$
Method:	Elecsys 2- site (after PEG extraction)

¹ R C Turner, N W Oakley and J D N Nabarro, BMJ. 1971: Vol 2, p 132 - 135

Interpretation

Raised values (ie increased insulin/glucose ratio)

Obesity, insulinoma, Cushing's Syndrome, Acromegaly, sulphonylurease, sex steroids, amino acids, glucagon and gut hormones, β blocking drugs, insulin receptor abnormalities.

In the presence of normal or elevated plasma glucose, raised fasting insulin suggests insulin resistance.

If patient hypoglycaemic, an elevated fasting insulin is suggestive of inappropriate insulin excess secondary to endogenous hypersecretion, sulphonylurea use or exogenous insulin administration.

Low values – (ie decreased insulin/glucose ratio)

Diabetes mellitus, hypokalaemia, pheochromocytoma, restricted carbohydrate intake, β -blocking drugs, hypoglycaemia, streptozotocin, somatostatin.

Insulin Antibodies

This test is intended to be used to detect antibodies to exogenously administered insulin that are present in sufficient quantity to possibly affect diabetes control.

It is not intended for the detection of insulin autoantibodies (IAA).

The presence of antibodies is detected by assaying specimens both extracted and unextracted.

Tick "Insulin antibodies" on request form. 1ml of EDTA plasma required.

Insulin, Free

As all samples are PEG treated before assay, the routine assay result is in fact an estimate of "free" insulin.

Insulin, Bound; Insulin, Total

Please enquire about availability

Insulin Autoantibodies (IAA)

This test is no longer available in New Zealand

Leptin

Clinical Applications:

This test is no longer being offered as a clinical measurement. It is available only to researchers who have batches of samples.

Leptin is secreted from adipose tissue and appears to be part of a regulatory loop linking fat mass to food intake, energy expenditure and reproductive adequacy.

Please enquire about assay availability and/or for an Endolab New Study Form for research projects.

Sample Aliquot Requirements:

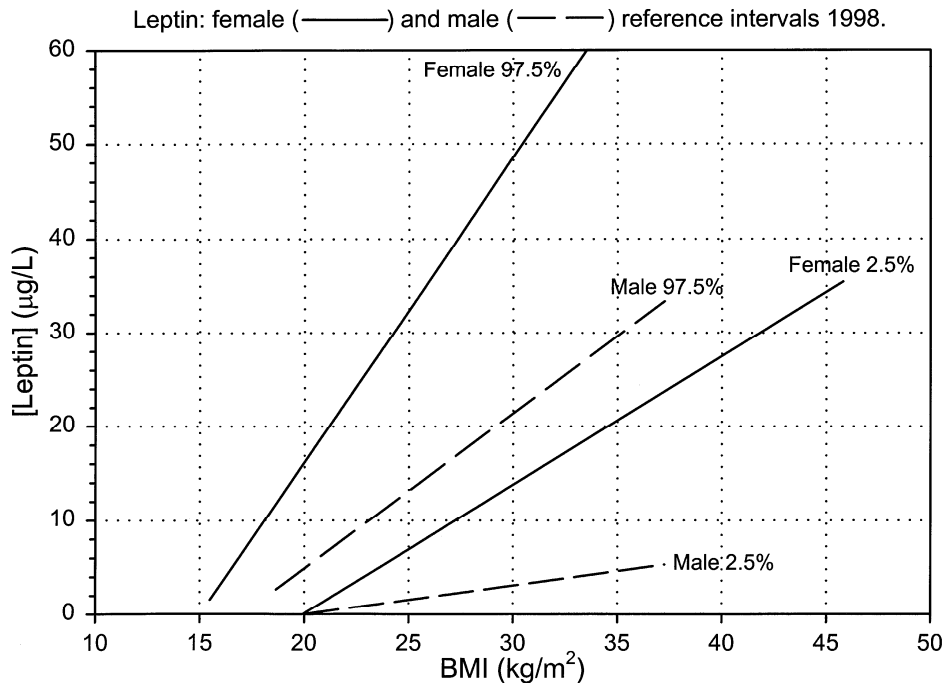
Laboratory	Endolab
Anticoagulant	EDTA
Volume	0.5ml plasma, minimum 0.25ml
Transport	Ambient overnight or to remain cold
Storage	$\leq -15^{\circ}\text{C}$
Species	Human

Test Details:

Frequency: By arrangement only.
Reference Interval: Related to BMI and gender, see graph below.

Female:	3-15 $\mu\text{g/L}$	(BMI = 20 kg/m^2)
	7-35 $\mu\text{g/L}$	(BMI = 25 kg/m^2)
Male:	1-3 $\mu\text{g/L}$	(BMI = 20 kg/m^2)
	2-10 $\mu\text{g/L}$	(BMI = 25 kg/m^2)

Standard: Recombinant human Leptin
Method: Linco RIA kit



LH, Luteinising Hormone – plasma, serum

Clinical Applications:

Investigation of primary and secondary amenorrhoea, and/or gonadal failure.

Assessment of hypothalamic-pituitary function. Note that repeated sampling may be necessary in order to demonstrate impaired LH secretion because of oscillations in LH values during the day. In postmenopausal women there is less difficulty, as resting values are higher than in pre-menopausal. A pre-menopausal value in postmenopausal woman is suggestive of hypothalamic-pituitary disease. In males a low plasma testosterone with low or “normal” LH values is suggestive of hypothalamic or pituitary disease.

Detection of pre-ovulatory surge – either spontaneous or in response to clomiphene citrate. A mid-cycle LH peak precedes ovulation by 24 – 48 hours.

Polycystic ovary syndrome – some but not all of these patients (approx. 50%) may have tonically increased plasma LH levels. (FSH is normal).

Patient Preparation

Time of sampling is not critical (except for some research studies). Details of age, gender, last menstrual period and medications, (especially oral contraceptive or sex steroids) are essential. To avoid confusion with the mid-cycle LH peak it may be necessary to take two samples at least two days apart.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	Serum or lith. heparin plasma (EDTA plasma acceptable but results may be 5% lower)
Volume	0.5ml serum or plasma, minimum 0.2ml serum or plasma
Transport	Ambient
Storage	≤-15°C
Species	Human

Test Details:

Frequency:	Daily (Monday – Friday)	
Reference Intervals:	0-9yrs	0-2.5 IU/L
	approx 10-15yrs	“Prepubertal range 0-2.5IU/L. Levels rise during puberty towards adult range”.
	Adult male	2-9 IU/L
	Adult female Follicular	2-8 IU/L
	Mid Cycle	10-75 IU/L
	Luteal	2-8 IU/L
	Post Menopause	>15 IU/L
	Antenatal	<1 IU/L
Standard:	2 nd IRP (80/552)	
Method:	Access Analyser, Beckman/Coulter	

Interpretation:

During the menopausal transition, which may last several months or longer, values of LH may fluctuate considerably.

Macroprolactin

Clinical application:

To distinguish hyperprolactinaemia that is caused by elevated monomeric (or “free”) prolactin from apparent hyperprolactinaemia that is caused by a raised macroprolactin content that interferes in the measurement of prolactin. Macroprolactin is considered to be biologically inactive. Other interferences for example rheumatoid factors may also cause spuriously raised prolactin levels.

Availability

This test is available as a separate test, however it is performed routinely in association with the measurement of prolactin(free) on all samples that are found by Endolab to have a raised prolactin level. Refer to the page on Prolactin (see page 83).

Sample aliquot requirements:

Laboratory	Endolab
Anticoagulant	serum or lith. heparin plasma. EDTA is acceptable only if the blood draw was complete
Volume	0.8ml serum/plasma, minimum 0.4ml serum/plasma
Transport	ambient
Storage	≤-15°C
Species	human

Test details

Frequency: twice weekly

Result interpretation: ND = not detected
L = low levels probable
H = mod-high levels

Method: Measurement of prolactin using the Beckman Coulter Access assay before and following PEG treatment. The test is not specific for macroprolactin and may also identify presence of other large molecular sized interferences.

Information

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Endolab

Christchurch Hospital

Private Bag 4710

Christchurch 8140

Phone: (03) 364 0848

Fax: (03) 364 0818

Toll free: 0800-Endolab (3636 522)

E-mail: endolab@cdhb.govt.nz

Refer to <http://www.cdhb.govt.nz/chlabs/endo/mprl.htm>

Neurotensin

Availability:

This assay is only available by sending the sample to the United Kingdom at a cost of around NZ\$400.00.

Sample Requirements:

Prepare trasylol tubes: Place 200µl sterile Trasylol.² (Bayer 10,000 KIU aprotinin/ml) in a commercial heparin blood tube. It is suggested that a large batch of Heparin + Trasylol tubes be made up at one time and stored in a refrigerator or freezer for future use.)

Take 10ml blood from a fasting patient.

Add blood to tube and mix gently by inversion.

Centrifuge immediately.

Separate plasma without haem. (If haemolysed repeat sampling).

Lyophilisation:

Measure separated plasma into aliquots of eg 1ml or 2ml (state volume for reconstitution purposes!!) and place in -20°C deep freeze within 15 minutes of venepuncture.

Freeze dry samples in vacuum until all water content has been removed. Seal in vacuum or under dry N₂.

Package carefully to avoid breakage and send with patient's clinical details and the sample reconstitution volume to:-

Professor S R Bloom
2nd Floor Francis Fisher Laboratories
RPMS Hammersmith Hospital
Du Cane Road
London W12 ONN
United Kingdom
Telephone: 44 181 740 3949
Fax: 44 181 740 3142

² Trasylol usually available via pharmacy as 5000,000KIU (50ml)

Estradiol, Oestradiol, 17 β - Oestradiol, E2 – plasma

Clinical Applications:

Assessment of ovarian function.

Assessment of ovarian response in infertile women treated with gonadotrophins or gonadotrophin releasing hormone. (Results should be interpreted in association with ultrasound findings).

Investigations of precocious puberty.

Investigation of patients with oestrogen producing tumours.

Patient Preparation

No special preparation. Oestradiol levels vary over the day with higher values in the morning.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA, heparin or fluoride preferred. Serum accepted but values may be higher (11%)
Volume	0.4ml plasma, 0.15ml minimum
Transport	Ambient
Storage	Frozen
Species	Most, except levels very low in some

Test Details:

Frequency:	2x weekly		
Reference Interval:	males:		40 – 110pmol/L
	females: (pre-menopausal, no OC)	follicular:	90-185pmol/L
		mid-cycle:	550-1650pmol/L
		luteal:	550-845pmol/L
	(post-menopausal, no HRT)		< 50pmol/L
Standard:			
Method:	DiaSorin Estradiol-2 RIA		

Oestriol (Estriol), (E3) – urine, serum

Availability:

This test is not routinely available in New Zealand as a clinical test. Inquire about availability for research purposes.

This test is not done by most world laboratories.

Oestriol by itself is not a very useful test, but may be helpful in cases of suspected Sulphatase deficiency. Measurement of unconjugated Estriol is sometimes used in a pre-natal triple-market screen for Down-Syndrome risk assessment.

Further information:

Dr Jane Ellis

Endolab

Christchurch Hospital

Phone: (03) 364 0886

Fax: (03) 364 0818

Freephone: 0800-endolab (36 36 522)

E-mail: endolab@cdhb.govt.nz

Oestrogens (non pregnancy) – urine

This test is no longer available.

Alternative tests suggested are:

oestrone-3-glucuronide, urine (page 75)

Oestrone-3-Glucuronide - urine

Clinical Applications:

Assessment of luteal function in the diagnosis and treatment of infertility in conjunction with pregnanediol-3-glucuronide.

Investigation of menstrual irregularity and the adequacy of corpus luteum development.

Patient Preparation

Plasma pregnanediol-3-glucuronide is measured instead of progesterone as it offers a better window for clinical interpretation. See Pregnanediol-3-glucuronide page 79.

Free urine ovulation profile kit available on request from:

Steroid Unit
Canterbury Health Laboratories
P O Box 151
Christchurch 8140
Fax line: (03) 364 0889
Telephone: (03) 364 0888

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	
Volume	5ml, store frozen. Sample should be an early morning void from menstruating women every fourth day from the start of menstruation until the cycle finishes. Approximately eight samples should be taken.
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	
Reference Interval:	Normal excretion for Women: Post Follicular, >30 nmol/24hr Follicular, <20 nmol/24hr
Standard:	
Method:	In house ELISA

Interpretation

Low excretion rates, < 30 nmol/day, are found in the early follicular phase of the menstrual cycle ie days 1 – 8. A midcycle peak, > 40 nmol/day, followed by a fall in value and a subsequent rise, together with pregnanediol values indicate progression to the luteal phase and ovulation.

See Pregnanediol-3-glucuronide (page 79) for further information.

Osteocalcin

Please enquire about availability.

At present (2009), this assay is available for batches of 20 or more samples for research projects. Please enquire for more details. Prices and turn-around times will vary depending on study conditions. Clinical samples may be assayed from time to time

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA or none
Volume	0.5ml plasma or serum
Transport	Frozen or liquid at 0 – 4°C
Storage	Frozen
Species	Human

Test Details:

Frequency:	Enquire
Reference Interval:	
Standard:	
Method:	Roche Elecsys 2010

Contact:

Dr J H Livesey
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: 0800-endolab (3636 522)
E-mail: endolab@cdhb.govt.nz

P1NP

Please enquire about availability.

The P1NP (total procollagen type 1 aminoterminal propeptide) assay is offered by arrangement for research purposes only.

Applications:

P1NP is a bone formation marker. It is a specific indicator of type 1 collagen deposition. It is released as a trimeric structure, but degrades to a monomer. The Elecsys assay detects both, ie. total P1NP.

Sample aliquot requirements:

Laboratory:	Endolab
Anticoagulant:	EDTA, Li-heparin or none
Volume:	0.9ml ideal, 0.3ml minimum
Transport:	for EDTA plasma, up to 1 day at ambient temperature, 5 days cold, otherwise frozen
Storage:	Frozen
Species:	Human, enquire about others

Test details:

Frequency:	by arrangement
Standard:	proprietary (Roche)
Method:	Roche Elecsys 2010 two-site electro-chemiluminescence immunoassay

Contact:

Dr Jane Ellis
Endolab
Christchurch Hospital
Phone: (03) 364 0886
Fax: (03) 364 0818
Freephone: 0800-endolab (36 36 522)
E-mail: endolab@cdhb.govt.nz

Pancreatic Polypeptide

This test is available irregularly. Please inquire.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA plasma
Volume	1ml plasma, 420µl minimum
Transport	EDTA Ambient All others cold or frozen.
Storage	Frozen
Species	

Contact:

Dr J H Livesey
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: 0800-endolab (3636 522)
E-mail: endolab@cdhb.govt.nz

Pregnanediol-3-Glucuronide - urine, plasma

Clinical Applications:

Assessment of luteal function in the diagnosis and treatment of infertility in conjunction with oestrone-3-glucuronide.

Investigation of menstrual irregularity and of the adequacy of corpus luteum development.

Patient Preparation

Sample should be an early morning void from menstruating women every fourth day from the start of menstruation until the cycle finishes. Approximately eight samples should be taken.

Plasma pregnanediol-3-glucuronide is measured instead of progesterone as it offers a better window for clinical interpretation. See Oestrone-3-glucuronide page 75.

Free urine ovulation profile kit available on request from:

Steroid Unit
Canterbury Health Laboratories
P O Box 151
Christchurch 8140
Telephone: (03) 364 0888
Fax: (03) 364 0889

Sample Aliquot Requirements:

Laboratory	Steroid Lab	
Anticoagulant		
Volume	Urine:	5ml, store frozen
	Plasma:	0.5ml, plasma or serum
Transport	Urine:	ambient
	Plasma:	ambient
Storage	Urine:	frozen
	Plasma:	ambient
Species		

Test Details:

Frequency:	Daily		
Reference Interval:	urine luteal	>5 $\mu\text{mol}/24 \text{ hr}$	
	follicular	<3 $\mu\text{mol}/24\text{hr}$	
	plasma, luteal,	>200nmol/L	
Standard:			
Method:	ELISA		

Interpretation

Unvarying low excretion rates are found in anovulatory women with high steadily increasing rates in pregnant women. Ovulation is followed by an increase in pregnanediol output, (greater than 5 $\mu\text{mol}/24 \text{ hr}$).

See also Progesterone - Plasma (page 81) or Oestrone-3-glucuronide (page 75) for further information.

Progesterone, 17 α Hydroxyprogesterone

serum/plasma

Clinical Applications:

In disorders of adrenocortical biosynthesis, particularly congenital adrenal hyperplasia (CAH). Samples should be taken in early morning (eg 0800h) if “non-classic” CAH is suspected.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, heparin
Volume	0.2ml serum or plasma, minimum 0.1ml Plasma preferred from infants greater than 5 days old for diagnostic purposes, as samples from infants less than 5 days old have variable elevated levels.
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	<15nmol/L
Standard:	
Method:	ELISA

Interpretation

High values are indicative of adrenal enzyme defect (21 or 11 β -hydroxylase deficiencies) or severe illness, occasionally raised in patients with adrenal or ovarian tumours.

Note: Other tests in which CAH may be useful if the diagnosis is in doubt are plasma 11-deoxycortisol, testosterone, DHAS, androstenedione, ACTH, renin. Minor adrenal enzyme deficiencies may become apparent following ACTH stimulation.³

³ Chetkowski R J et al, J Clin Endocrinol Metab; 1984; 58: 595

Progesterone - serum, plasma

Clinical Applications:

Assessment of occurrence of ovulation and corpus luteum function.

Patient Preparation

No special preparation but blood sample should be taken from 6 to 9 days following the presumed day of ovulation. A brief summary of clinical particulars, including any relevant drug therapy eg clomiphene, should be provided. Please state time of sampling and day of menstrual cycle.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, heparin, plain
Volume	0.5ml plasma, minimum 0.2ml
Transport	Ambient
Storage	Ambient
Species	

Test Details:

Frequency:	3-4 times per week
Reference Interval:	Follicular phase: < 15nmol/L Luteal phase: > 15nmol/L
Standard:	
Method:	ELISA

Progesterone - salivary

Salivary progesterone is also offered, which has applications to women using progesterone supplements during menopause.

Sample requirements:

1.0 - 2.0ml of saliva collected in a sterile container. Collection kit is available from the laboratory.

Transport:

Ambient

Interpretation

The optimal level of salivary progesterone in post-menopausal women using transdermal progesterone cream is 3-10nmol/L, although this is open to debate.

Values grossly exceeding this are not uncommon and probably arise from contamination.

Post-menopausal women not on replacement have values similar to the follicular phase, namely <0.5nmol/L.

Peak luteal phase salivary progesterone is >1.5nmol/L.

Contact:

Dr John Lewis

Steroid Biochemistry

Canterbury Health Laboratories

Phone: (03) 364 0888

Fax: (03) 364 0889

Prolactin (Prl) and “free” Prolactin (FPRL)

Clinical Applications:

Investigation of galactorrhoea.

Investigation of amenorrhoea, infertility or hypogonadism. Raised prolactin levels are (often without galactorrhoea) associated with impaired gonadal function.

Differential diagnosis of pituitary fossa enlargement. Many "non-functioning" pituitary tumours in males and females are prolactin-secreting adenomas. Prolactin estimations are important in assessing the response to pituitary surgery, radiotherapy and drugs such as bromocriptine or cabergoline.

Assessment of hypothalamic-pituitary function. Basal prolactin concentrations may be raised in some patients with hypothalamic disorders such as craniopharyngioma. TRH and chlorpromazine stimulation have been used to further demonstrate abnormalities in prolactin secretion. The results are variable, and these tests are not recommended as a routine.

Specific Instructions

Patient should be unstressed but need not be fasting. Morning samples preferred.

It is essential to state the medication that the patient is receiving, especially phenothiazine derivatives, aldomet, metoclopramide and oral contraceptive preparations etc.

A statement of the menstrual history and presence of absence or galactorrhoea is also necessary.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	serum or lith. heparin plasma. EDTA is acceptable only if the blood draw was complete
Volume	0.8ml serum/plasma, minimum 0.4ml serum/plasma
Transport	ambient
Storage	≤-15°C
Species	human

Test Details for Prolactin:

Frequency:	daily (Monday - Friday)	
Reference Interval:	Female (non pregnant)	50-550 mIU/L
	Male	50-350 mIU/L
Standard:	3rd IS 84/500	
Method:	Access Analyser, Beckman/Coulter	

All samples with prolactin results above the reference intervals will automatically be screened for macroprolactin presence.

Interpretation

Refer to <http://www.cdhb.govt.nz/chlabs/endo/mprl.htm>

Hyperprolactinaemia may result from hypothalamic and pituitary disorders, pregnancy, primary hypothyroidism, chronic renal failure, cirrhosis and polycystic ovary syndrome, drugs such as anti-psychotics, antiemetics (eg. metoclopramide), antidepressants, opiates and antihypertensives (methyldopa, reserpine, and verapamil). Spurious hyperprolactinaemia can occur because of cross-reaction or interferences in a prolactin assay; for example by macroprolactin, heterophile antibodies or rheumatoid factors.

A level of prolactin >5000 is highly suggestive of a prolactinoma.

This method has relatively low interference from macroprolactin, however, samples can be tested for “free” (monomeric) prolactin and a comment on the likelihood of macroprolactin presence issued. Current consensus opinion is that patients with macroprolactinaemia and a normal “free” prolactin, who do not have clinical features of hyperprolactinaemia or a pituitary mass lesion do not warrant further investigation eg. MRI imaging.

Changes in Prl levels may occur seasonally (highest in spring) and across the day (circadian rhythm); nocturnal surges have been reported.

Test Details for “free” Prolactin:

Frequency:	twice weekly
Standard:	3rd IS 84/500
Method:	Access Analyser, (Beckman/Coulter) following PEG pre-treatment

PRA - Renin Activity

Clinical Applications:

Aetiology of hypertension (primary hyperaldosteronism, and other mineralocorticoid excess states associated with high blood pressure, renal artery stenosis, renin secreting tumours). Best done in conjunction with measurement of plasma aldosterone (see page 19).

Localising renal ischaemic disease (renal vein renin sampling).

Diagnosis of primary adrenocortical insufficiency and assessing adequacy of mineralocorticoid replacement.

Diagnosis of Bartter's syndrome.

Diagnosis of hypovolaemic disorders (eg patients presenting with hyponatraemia, postural hypotension etc).

Diagnosis of hyporeninaemic syndromes.

Patient Preparation

Patient's posture, sodium intake, drug therapy and time of sampling all profoundly alter PRA.

Outpatients are best screened as follows:

If possible stop non-essential drugs for 2 weeks before sampling. Many hypotensive drugs alter renin levels; diuretics and ACE inhibitors increase PRA, whereas Beta-blockers reduce PRA. Alpha-blockers (Doxazosin, Prazosin and related drugs) or calcium channel blockers have less effect and are therefore preferred where the clinical condition allows.

Patients should attend (non-fasting) prior to 10.00 am for "ambulant" sampling for PRA. Plasma aldosterone (see page 19) is also usually necessary for interpretation.

Inpatients are also screened as above and should be ambulated for at least 30 minutes before sampling. "Bed bound" patients may be sampled after a similar time in sitting position.

Other protocols involving PRA measurement include frusemide challenge, 2 hours of quiet standing or response to sodium depleting diets. Consult with an Endocrinologist for indications and test protocols.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA plasma
Volume	0.5ml plasma, minimum 0.3ml
	Blood centrifuged within 30 minutes at 4°C and plasma stored and transported deep-frozen. Thawed samples will not be assayed.
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	Weekly
Reference Interval:	0700 - 1100 ambulant: 0.4 - 2.3nmol/L/hr
Standard:	
Method:	Enzymatic RIA

Interpretation (refer also to aldosterone-renin ratio, see page 22)

Low PRA - Primary aldosteronism and other mineralocorticoid excess syndromes; "low renin" hypertension; in the aged population.

High PRA - Adrenocortical insufficiency, fluid and salt wasting syndromes; Bartter's syndrome, severe renal ischaemia, malignant hypertension, renin secreting tumours, and in young children.

Plasma aldosterone/PRA ratio greatly assists interpretation, particularly in primary hyperaldosteronism.

Details regarding drug therapy, (including oral contraceptives) and 24 hr urine electrolyte excretion will aid interpretation. Please state time and posture prior to sampling.

For details of availability and specimens required, please contact:

Dr T G Yandle

Endolab

Christchurch Hospital

Private Bag 4710

Christchurch 8140

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PTH, Parathyroid Hormone

Clinical Applications:

Investigation of abnormalities of calcium or phosphate homeostasis, such as hypercalcaemia, hypocalcaemia, hyperparathyroidism and hypoparathyroidism.

The PTH immunometric assay is specific for the intact PTH molecule. Inactive carboxy-terminal fragments which accumulate in renal failure do not cross-react in this assay which separates normal subjects from those with primary hyperparathyroidism though there is a small overlap. The assay is sufficiently sensitive to give low values in patients with non-parathyroid hypercalcaemia (eg due to malignancy) and there is no known cross-reaction with PTHrP in non metastatic malignant hypercalcaemia.

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	0.9ml EDTA plasma, Minimum: 0.3ml EDTA plasma. Separate blood within two hours of collection. Serum calcium, phosphate and creatinine should be requested and collected at the same time as the sample. If transported frozen, serum is also acceptable.
Transport	Ambient <24 hours, otherwise frozen or in iced water.
Storage	Frozen
Species	Human

Test Details:

Frequency:	daily
Reference Interval:	1.6 – 7.0pmol/L (pg/ml = 9.4 x pmol/L)
Standard:	1-84hPTH
Method:	Elecsys sandwich electro-chemiluminescence immunoassay

PTHrP, Parathyroid Hormone Related Peptide

Clinical Applications:

May be of value in the differential diagnosis of hypercalcaemia.

PTHrP is often raised in hypercalcaemia of malignancy, whereas PTH is infrequently elevated in this condition.

PTHrP is not elevated in primary hyperparathyroidism whereas PTH usually is.

Patient Preparation

PTHrP is rapidly destroyed by plasma enzymes. Blood must be collected and then mixed in a special tube containing a cocktail of enzyme inhibitors.

Sample Aliquot Requirements:

Laboratory	Endolab - Christchurch
Anticoagulant	Special PTHrP cocktail tube containing enzyme inhibitors. Available for use outside of Christchurch by arrangement, from Endolab, Christchurch Hospital. The special tube must be stored frozen until use.
Collection	<p>In Christchurch, samples for this test must only be taken by the Endocrine Test Centre, Ward 26, Christchurch Hospital because of the limited shelf life of these tubes and the instability of the sample.</p> <p>The technique is to draw the blood sample with a syringe & needle and then transfer about 3ml of the blood from the syringe to the white-topped PTHrP cocktail tube, mix the contents gently with 4 or 5 inversions. Keep the tubes in iced water for transport within the hospital. The tube is then centrifuged and the plasma transferred to the screw-cap plastic sample tube (labelled "PTHrP plasma only") provided. It should then be stored and transported frozen.</p>
Volume	0.5ml of plasma is needed for the assay.
Transport	Frozen
Storage	Frozen

Test Details:

Frequency:	Irregularly – usually every 6 months – please enquire if required more urgently, which can often be provided at the cost of a new kitset at around NZ\$1,000.00.
Reference Interval:	Interim: <1.5pmol/L
Standard:	Synthetic hPTHrP 1-87
Method:	Mitsubishi IRMA

SHBG, Sex Hormone Binding Globulin

Clinical Applications:

Hirsutism and virilisation.

Monitoring male cancer patients on oestrogen therapy.

Note: Normally done in conjunction with a request for plasma testosterone.

Patient Preparation

No special preparation. Please state time of sampling, and when applicable day of menstrual cycle and nature of any drug therapy. Plasma levels of SHBG rise in pregnancy and are elevated in women taking oestrogen dominant oral contraceptives. Please provide brief summary of clinical particulars.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, heparin, plain
Volume	0.5ml serum or EDTA plasma, 0.2ml minimum
Transport	Ambient
Storage	
Species	

Test Details:

Frequency:	daily
Reference Interval:	♂, < 10 - 60nmol/L ♀, 20 - 90nmol/L
Standard:	
Method:	In house ELISA

Interpretation

SHBG is a serum protein that binds testosterone, oestradiol-17 β and closely related steroids with a 17 β -ol configuration. While bound to SHBG, the steroid cannot express its hormonal action. Usual assays for testosterone give a measurement of the total (free + bound) concentration of the hormone. When SHBG is also determined a measure of the free and active concentration of the hormone is provided. In a study of 136 hirsute women 38% were found to have plasma SHBG capacity levels below the lower limit for normal women indicating, as a consequence, elevated concentrations of free testosterone. This is a controversial subject and considerable debate exists over what constitutes the "free" fraction of steroid hormone.

See Carlstrom et al, Gynecol. Obstet. Invest., Vol 24, 256-261, 1987.

Mooradian et al, S Steroid Biochem., Vol 29(3), 369-370, 1988.

See comments under testosterone regarding FAI and free testosterone.

Somatostatin, GHRH

Research only, please inquire

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	3ml EDTA plasma from fasting patient, 0.6ml minimum Collect 5ml blood into an EDTA tube in iced water. Separate without delay and deep freeze plasma in two tubes.
Transport	Frozen Send in a dry-ice container to: Endolab, 2nd Floor, Riverside Block, Christchurch Hospital, Private Bag 4710 Christchurch 8140
Storage	Frozen
Species	

Testosterone - serum, plasma, saliva

Clinical Applications:

Diagnosis of primary and secondary (pituitary) forms of gonadal failure in post pubertal male subjects.
In the evaluation of pubertal development of male adolescents - particularly in those demonstrating delayed or arrested development.
In states of precocious puberty in either sex.
In hirsute and/or virilised females.
In the syndrome of "testicular feminisation".

Patient Preparation

No special preparation. Plasma testosterone levels may fluctuate over the day, with higher values in the forenoon. A preference for sampling between 0800-0900 hr exists, since better comparison with normal subjects is then available. Please state time of sampling.

Additional Tests: employing dexamethasone suppression, HCG stimulation etc, may be helpful in some clinical circumstances.

SHBG should also be measured with testosterone.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	serum, EDTA, heparin
Volume	0.5ml serum or plasma, minimum for males 0.3ml, for females 0.5ml 1-2ml saliva in a sterile container
Transport	Ambient
Storage	Ambient
Species	

Test Details:

Frequency:	daily
Reference Interval:	Plasma: adult ♂, 9 - 38nmol/L adult ♀, 0.5 - 2.7nmol/L
	Saliva:
Standard:	
Method:	ELISA

Interpretation

Low Levels are seen in children, delayed male puberty and in adult males with hypogonadism.

High Levels are seen in females with virilising disorders and in most adult females with severe hirsutism.

Oestrogen therapy and pregnancy will raise testosterone values.

Elevated values may also occur in hyperthyroidism.

Considerable debate arises as to what constitutes the "free" hormone. Evidence exists that albumin bound hormone is also free. Concentrations of free fatty acids also have affects on binding of testosterone.

The free androgen index (FAI) and free testosterone (pmol/L) are calculated from total testosterone and sex-hormone binding globulin levels.

	♀	♂
FAI	<80	>400
Free T	<50	250-800

Thyroglobulin (Tg)

Clinical Applications:

Thyroglobulin (Tg), the precursor protein for thyroid hormone synthesis is detectable in the serum of most normal individuals when a sensitive assay method is used. The serum Tg level integrates three major factors (1) the mass of differentiated thyroid tissue present (both normal and neoplastic) (2) any inflammation or injury to the thyroid which causes release of Tg (3) the amount of stimulation of the TSH receptor (by TSH, TRAb or hCG). An important issue is the potential for thyroglobulin autoantibodies (TgAb), which are detected in a higher percentage of patients with differentiated thyroid carcinoma (DTC) than the general population (20 vs. 10% respectively), to result in underestimation of the Tg value in patients with DTC. Both Tg and TgAb will therefore be measured on each sample sent to the laboratory – the detection of TgAb, irrespective of titre, has the potential to produce a falsely low Tg result and the Tg result should thus be cautiously interpreted in these patients.

Patient preparation:

Pre-operative specimens should be taken before fine needle aspiration (FNA) or >2 weeks after FNA.

Sample aliquot requirements:

Laboratory	Endolab
Anticoagulant	serum, heparin
Volume	0.5ml serum or heparin plasma
Transport	Ambient <8hours otherwise cold/frozen
Storage	Frozen
Species	Human

Test details:

Frequency	weekly
Reference interval	0-58µg/L. Tg should be undetectable (<0.1µg/L) in a patient who has had a total thyroidectomy and has a TSH <0.1mIU/L.
Standard	CMR457
Method	ICLMA on the Beckman Coulter Access Analyser

Interpretation:

Differentiated thyroid carcinoma (DTC). An elevated pre-operative serum Tg level is seen in 2/3 patients with DTC indicating that these tumours have the capacity for Tg secretion and by inference, post-operative Tg monitoring can be clinically useful in these patients. If the pre-operative serum Tg level is within normal limits, an undetectable post-operative serum value is less reassuring because it is unclear whether the tumour originally secreted Tg. The sensitivity of post-operative serum Tg monitoring for detecting recurrence will be highest when the tumour is relatively small (<2cm diameter) and the pre-operative Tg is high – note that pre-operative specimens should be taken before fine needle aspiration (FNA) or >2 weeks after FNA.

Tg measured 2 months after surgery reflects the amount of residual normal thyroid tissue plus any tumour remnant provided that TSH is suppressed below 0.1mU/L. Since the thyroid remnant after near total thyroidectomy typically approximates 2 grams of tissue, a serum Tg concentration of <2µg/L is expected (since one gram of normal thyroid tissue produces approx. 0.5 µg/L @ TSH <0.1 mIU/L) after near total thyroidectomy.

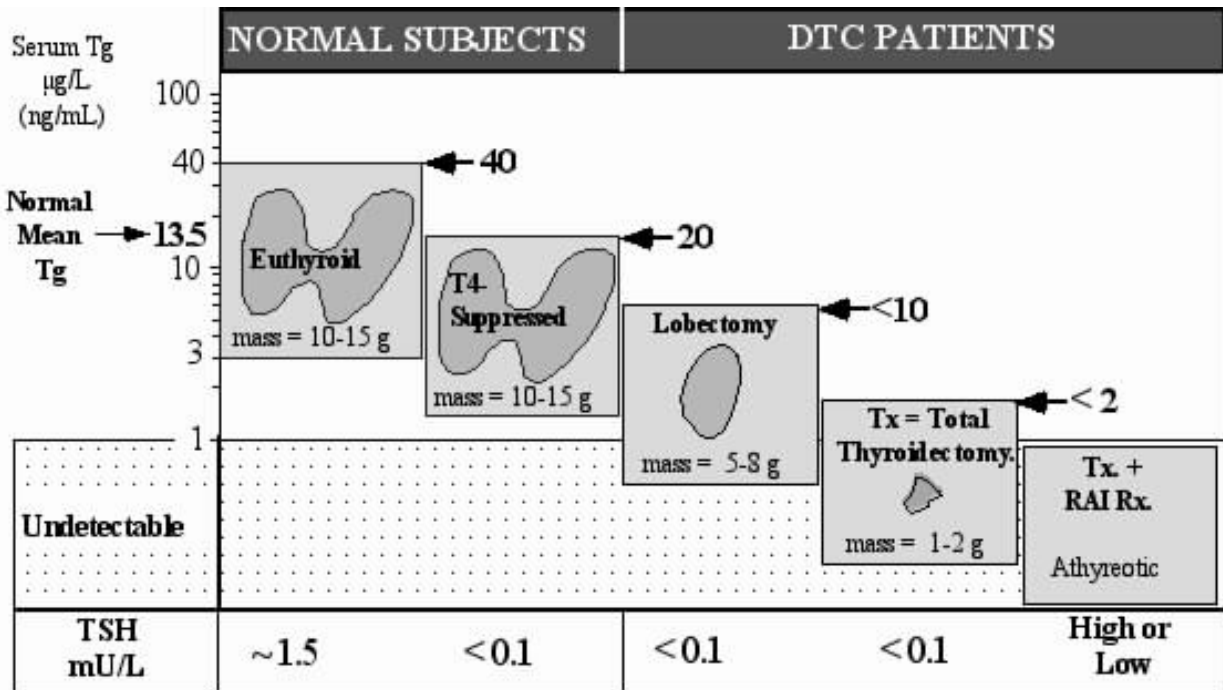
Serum Tg is frequently measured during long term monitoring of patients with DTC treated with suppressive Thyroxine therapy. In this situation, clinical recurrence in tumours judged to be “poor Tg secretors” (normal range pre-op Tg) may be associated with low or undetectable post-operative Tg values. In contrast, recurrence of tumours considered as “good Tg secretors” (elevated pre-op Tg) is usually associated with a progressive rise in serum Tg. The pattern of serial Tg measurements, made when the patient has a stable TSH, is more clinically useful than an isolated Tg value. However, it is possible to interpret an isolated Tg value by knowing the normal reference range of the Tg assay, the extent of thyroid surgery and the steady state TSH level, as shown in the Figure below.

Expected serum Tg values relative to thyroid mass and TSH status. Assumptions include: (1) no recent thyroid surgery or FNA, (2) mass of normal thyroid tissue = 10-15 grams, (3) one gram of normal thyroid tissue produces approx 1µg/L Tg in serum @ normal TSH and (4) one gram of normal thyroid tissue produces approx 0.5 µg/L Tg in serum @ TSH <0.1 mIU/L.

Non-neoplastic conditions: Serum Tg is elevated in the majority of hyperthyroid states – a low Tg can be a useful indicator of thyrotoxicosis factitia, characterized by a non-elevated serum Tg in a biochemically toxic patient. Patients with thyroiditis (e.g. subacute thyroiditis or amiodarone-induced thyroiditis) have increased serum Tg values in the acute phase of the Thyrotoxic State.

Reference:

NACB – Laboratory support for the diagnosis and monitoring of thyroid disease. Demers LM, Spencer CA 2002. (www.nacb.org)



Thyroid Function Tests

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	
Volume	1.5ml serum, includes full thyroid function tests, minimum 1.0ml.
Transport	Ambient
Storage	
Species	

T4 Total, Thyroxine - serum

Clinical Applications:

Measurement of serum total T4 is the most useful routine thyroid function test in the diagnosis of thyrotoxicosis or hypothyroidism. The calculation of a free thyroxine index adjusts the serum thyroxine for changes in the serum proteins where total serum T4 may not give a good index of thyroid function, e.g. during oral contraceptive therapy or pregnancy a serum thyroxine is often significantly elevated, but the calculated free thyroxine index is normal. Conversely, in patients with subnormal serum proteins the serum thyroxine is often low but the free thyroxine index is normal.

Test Details:

Frequency:	Daily (Monday - Friday)
Reference Interval:	55 - 140nmol/L
Standard:	Sigma
Method:	RIA

T3 Total, Triiodothyronine - serum

Clinical Applications:

The measurement of the total serum T3 has a very limited use in patients where a diagnosis of thyrotoxicosis is suspected but the serum T4 is high normal or only marginally elevated. Total serum triiodothyronine levels fall with age so that the value may be in the high normal range in thyrotoxicosis in the elderly. Calculation of a "free T3 index" using a measurement of thyroxine binding globulin may also be necessary.

Test Details:

Frequency:	2x weekly
Reference Interval:	1.2 - 2.8nmol/L
Standard:	Sigma
Method:	RIA

Interpretation

Elevated values - thyrotoxicosis, thyroxine or amiodarone therapy, and severe illness.

Subnormal values - hypothyroidism, hypopituitarism, lithium therapy, and severe illness.

Note: The total serum triiodothyronine level in particular may be subnormal in patients with chronic or acute severe non-thyroidal illness and in malnutrition.

TSH, Thyroid Stimulating Hormone - serum

Clinical Applications:

The diagnosis of hypothyroidism.

To separate primary thyroid failure from hypopituitarism. In thyroid deficiency the TSH is elevated whereas in pituitary-hypothalamic disease the TSH is usually normal or subnormal.

In association with Thyrotrophin Releasing Hormone stimulation tests.

Diagnosis of thyrotoxicosis.

Assessment of replacement thyroxine therapy.

Test Details:

Frequency:	Daily (Monday - Friday)
Reference Interval:	0.40 – 4.00mU/L

Standard: IRP 80/558
Method: Hypersensitive TSH, Access (Beckman Coulter)

Interpretation

1. In primary hypothyroidism the TSH level is elevated.
2. Suppressed TSH levels occur in thyrotoxicosis, thyroid autonomy, nodular goitre, past history of thyrotoxicosis.
3. After thyroid surgery or radioiodine therapy, TSH may be elevated despite clinical euthyroidism. The elevated TSH level is a warning that thyroid hormone concentration is sub-optimal. Such patients require either -
 - Thyroxine replacement therapy, or
 - Regular follow up to detect the development of clinical thyroid deficiency.
4. TRH-stimulation tests: See page 114

Vitamin D, 1,25-Dihydroxyvitamin D 1,25(OH)₂D

This test is no longer performed in New Zealand, but samples can be sent to Australia via Canterbury Health Laboratories.

Vitamin D, Hydroxycholecalciferol (25(OH)D)

Clinical Applications:

Serum levels of 25(OH)D are the best indicator of vitamin D status, either deficiency (which may result in rickets/osteomalacia), or excess (e.g. following inappropriate use of vitamin D supplements, and which may result in hypercalcaemia). It is of no value in assessing patients taking calcitriol (1,25(OH)₂D).

Levels of 1,25(OH)₂D may under special circumstances be helpful in replacement therapy or treatment of hypoparathyroidism with 1,25(OH)₂D. Although 1,25(OH)₂D is no longer available in New Zealand, samples can be sent overseas.

Sample Aliquot Requirements:

Laboratory	Steroid Lab
Anticoagulant	EDTA, heparin, plain
Volume	0.5ml, minimum 0.2ml
Transport	Frozen
Storage	Frozen
Species	

Test Details:

Frequency:	3x weekly	
Reference Interval:	<25nmol/L	moderate to severe Vitamin D deficiency
	25-50nmol/L	Vitamin D deficiency
	50-150nmol/L	optimal target range for bone health
	>200nmol/L	possible toxicity
Standard:	25(OH)D	
Method:	RIA	

VIP, Vasoactive Intestinal Peptide

Clinical Applications:

Investigation of watery diarrhoea (VIPoma).

Sample Aliquot Requirements:

Laboratory	Endolab
Anticoagulant	EDTA
Volume	2ml plasma or serum, minimum 1.0ml
Transport	Frozen or must remain cold (stable up to 5 days at 4°C)
Storage	≤-20°C
Species	Human, bovine, porcine, rat.

Test Details:

Frequency:	three weekly
Reference Interval:	<20pmol/L (adult, fasting). Values tend to be lower in the non-fasting state.
Standard:	h/p/r VIP ₍₁₋₂₈₎ Peninsula
Method:	RIA after plasma extraction

For details of availability, please contact:

Dr Jane Ellis
Endolab
Christchurch Hospital
Private Bag 4710
Christchurch 8140
Phone: (03) 364 0848
Fax: (03) 364 0818
Toll free: 0800-endolab (3636 522)

Dynamic Tests of Endocrine Function

1 mg Dexamethasone Suppression Test

Purpose of Test:

To differentiate normal subjects from those with Cushing's Syndrome.

Patient Preparation

No preparation. The patient need not be fasting. It is preferable for the patient to be off medication.

Procedure:

At midnight the patient takes 1.0 mg Dexamethasone orally. At 0800 hr the next morning, blood is drawn for plasma cortisol.

Interpretation

A plasma cortisol of less than 100nmol/L virtually excludes Cushing's Syndrome. Values above 100nmol/L should prompt further investigation. Values between 100 and 150nmol/L are a "grey area" and are unlikely to represent Cushing's syndrome, but require further assessment and discussion with an Endocrinologist. False positives may occur when patients are depressed, stressed, alcoholic or are receiving drugs such as phenytoin or chronic barbiturate treatment.

8mg Dexamethasone Suppression Test

Purpose of test

To differentiate between the two causes of ACTH-dependent Cushing's syndrome, namely pituitary-dependent Cushing's (Cushing's disease) and ectopic ACTH.

Patient preparation

No preparation, no need for fasting. Avoid use of drugs that increase Dexamethasone clearance eg. phenytoin

Protocol

Day 1 (a) take a sample for plasma cortisol at 0800h, 0830h and 0900h
(b) give Dexamethasone 8mg orally at 2400h

Day 2 take a sample for plasma cortisol at 0800h, 0830h and 0900h

Interpretation

The principle behind the test is that patients with a pituitary source for the ACTH in general have a greater degree of suppression of plasma ACTH and cortisol than occurs in patients with ectopic ACTH production. The original NIH criterion is that >50% suppression of 08.00 plasma cortisol on day 2 compared to day 1 suggests a pituitary source of ACTH. The more recent NIH criterion suggests that >68% suppression of plasma cortisol at 0900h on day 2 compared to 0830h on day 1 distinguish pituitary from ectopic ACTH with 100% specificity and 70% sensitivity.

References

Dicheck et al, 1994. JCEM 78: 418-22.

75 g oral glucose tolerance test for diabetes mellitus

Clinical applications

For diagnosis of diabetes mellitus, impaired glucose tolerance and assessment of insulin resistance.

Patient preparation

Fasting, ie. water only after 9pm the previous night.

Procedure

0' 0800 - 0900h basal blood sample

75 mg oral glucose given with straw over 2-3 minutes (= 225ml of 296ml solution containing 100gm dextrose).

120' blood sample

Blood samples assayed for glucose (and insulin where indicate).

Interpretation

Normal fasting plasma glucose	<5.6mmol/L 5.6 – 6.96mmol/L impaired fasting glycaemia ≥7.06mmol/L diabetes (on two occasions)
2 hour plasma glucose	≤7.86mmol/L normal 7.8 – 11.6mmol/L impaired glucose tolerance ≥11.16mmol/L diabetes mellitus
Normal fasting insulin	15 – 80pmol/L

GH Stimulation Tests

Arginine Infusion

Aim:

To test growth hormone release and, on rare occasions, insulin release (which has a separate indication). The test may, if desired, be followed by an insulin tolerance test (ITT) (See separate protocol, page 103)

Patient Preparation

Patient should be fasting overnight, but may drink water.

Contra-indications:

Certain drugs interfere with Arginine/Clonidine stimulation

Precautions:

Antihistamine and adrenaline should be available for treatment of potential allergic reactions to arginine. Excessive infusion rates can result in local irritation, flushing, nausea or vomiting.

Inadequate dosage (or prolongation of the infusion period) may result in diminished stimulus to the pituitary and nullification of the growth hormone reserve test.

Materials:

IV equipment, 1 bag 0.9% NaCl, giving set.

Arginine hydrochloride 5% in 500ml bottle (order through Pharmacy).

4 x 5ml EDTA tubes (clearly labelled with times).

Method:

Weigh patient.

Settle patient comfortably and give explanation.

Insert IV line.

Take -30 minute, then zero blood samples.

Keep vein open with 0.9% NaCl.

Infuse Arginine Hydrochloride 5% 11ml/kg over 30 minutes, allowing time to flush line with 0.9% NaCl before taking next sample.

Further samples then +30, +60 and +90 minutes.

Break fast with food and fluid as desired.

Samples:

Growth Hormone (1 - 3ml EDTA tube to Endolab)

Other basal bloods as ordered by doctor.

Interpretation:

Present normal cut off (as accepted for Health Department Childhood GH Advisory Committee) is 4µg/L (normal >7µg/L) (1µg/L = 2.7mU/L). Levels in childhood between 4 and 7µg/L suggest partial GH deficiency. The cut off is very arbitrary, but is the present local criterion. The same cut off applies to any physiologically or pharmacologically stimulated test.

Reference:

1974 - 1990 Micromedex Inc. Vol. 6

Clonidine Test

Aim:

Clonidine is a central α -adrenergic stimulator which induces GH release in normal children⁴, (see references) via stimulation of central adrenergic pathways. Release is impaired or absent in adults or children with hypopituitarism.

Patient Preparation

The patient should be fasting (overnight) and supine.

⁴ JCEM 41:827,1975

Procedure:

An IV line inserted and blood samples collected at:

-15, 0, +15, 30, 45, 60, 90 120 minutes.

Clonidine: 0.15 mg/m² is given orally at the time zero.

Measure lying BP at 60 and 120 minutes and when upright at the end of the test.

Blood samples:

The six samples are collected for growth hormone estimation and clearly labelled with time of sampling (see growth hormone for details of samples required). Other basal bloods as required by doctor.

Interpretation

Present normal cut off (as accepted for Health Department Childhood GH Advisory Committee) is 4µg/L (normal >7µg/L) (1µg/L = 2.7mU/L). Levels in childhood between 4 and 7µg/L suggest partial GH deficiency. The cut off is very arbitrary, but is the present local criterion. The same cut off applies to any physiologically or pharmacologically stimulated test.

Side Effects:

Mild fall in BP, care when walking after end of test.

Mild drowsiness for up to three hours.

References:

1. Gil-Ad, Topper and Laron: Oral clonidine as a growth hormone stimulation test. Lancet 1979: ii, 278-80.
2. Health Services hGH Committee. Comparison of the ITT and oral clonidine tolerance test for GH secretion, Arch Dis Child 1981: 56, 852-854.
3. Fraser N.A., Seth J, Brown N.S.: Clonidine is a better test for growth hormone deficiency than insulin hypoglycaemia. Arch Dis Child 1983:58, 355-358.

Glucagon test**Indications**

If ITT/Metyrapone testing is contraindicated in patients with possibly impaired HPA function. This is also a reliable test of GH release.

Preparations

Fast overnight. Prepare as for ITT.

Test

1 mg glucagon s.c. at zero time.

Take bloods at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min for glucose, cortisol (ACTH) and hGH.

Side effects

Nausea, vomiting.

Deterioration of blood sugar control in diabetics.

Interpretation

Cortisol should rise above 550nmol/L.

Present normal cutoff (as accepted for Health Department Childhood GH Advisory Committee) is 4µg/L (normal >7µg/L) (1µg/L = 2.7mU/L). Levels in childhood between 4 and 7µg/L suggest partial GH deficiency. The cutoff is very arbitrary, but is the present local criterion. The same cutoff applies to any physiologically or pharmacologically stimulated test.

Insulin Tolerance Test**Clinical Applications:**

To assess hypothalamic – pituitary – adrenal axis and growth hormone axis function. Clinical consultation with an Endocrinologist must precede the test being performed.

Purpose of Test:

To lower plasma glucose sufficiently to stimulate the secretion of growth hormone, cortisol and in some cases, ACTH. This requires a 50% drop from baseline values at 20 - 30 minutes post insulin and/or a fall of plasma glucose to 2.2 mmol or less.

Indications:

As a test of hypothalamic-pituitary function.

Side Effects:

Symptomatic hypoglycaemia 20 to 30 min after insulin administered. May provoke cerebral or cardiac complications - (very rare).

Patient Preparation:

Ideally an ECG should be done and inspected before the test. The patient is fasted for 8 hours overnight and rests in bed for 1 hour prior to the test beginning and continues to rest in bed throughout the test. The patient should not be receiving any drugs. If patient is on steroids or insulin, special arrangements regarding the doses of these hormones have to be made. Patient must not have consumed alcohol within 24 hr. The tests should not be done if veins are doubtful. At least one "good vein" is essential. The test should not be done in epileptics, in the very old (70 yrs), in patients with ischaemic heart disease or important rhythm disorders.

Dosage:

Soluble insulin is used as a rapid intravenous injection and if 20 units per ml solution is used, small doses can be dispensed with accuracy. In all cases the dose of insulin must be calculated and checked by the Registrar administering the insulin, after consultation with consultant Physician.

Dose schedule:

Likely Hypopituitary, underweight and not on replacement therapy, also for children under 4 yr age: 0.05 U/kilo body weight.

Possible Hypopituitary, children 4-8 years: 0.1 U/kilo.

Normals, children older than 8 y: 0.15 U/kilo; 0.2U/kilo if sex steroid primed.

Obese patients: 0.2 U/kilo.

Acromegalic or giants: 0.3 U/kilo.

Equipment:

1. 1 IV cannula, luer plug, syringes, tegaderm, needles, syringe with saline, Gelofusine.
2. Labelled tubes, observation chart, timing clock.
3. Normal saline, amps 50% Dextrose, drawing up cannula, labels and tubes for samples, insulin.

Insulin preparation 20 units/ml:**Equipment**

- 1 500ml bag Gelofusine
- 100 units/ml actrapid insulin (penpill)
- 1 3ml syringe
- 1x 1ml syringe, 1x 1 insulin syringe
- 3x needles

Method to make 20 units per ml of solution of insulin:

- Draw up 2ml of Gelofusine in 3ml syringe.
- Draw up 0.5ml (50 units) of Actrapid insulin into 1ml Insulin syringe .
- Add 0.5ml of Actrapid insulin to 2ml of Gelofusine in 3ml syringe.
- i.e. 50 units insulin in 2.5ml
- 20 units insulin/ml
- Draw required amount of insulin from the 20 unit/ml solution into the new 1ml syringe ready for injection.

Procedure:

1. Insert cannula into suitable vein. Take glucose sample and test. Patient rests 30 minutes. Before beginning test have insulin drawn up.
2. Draw zero sample.
3. Immediately after sampling Physician/RN gives IV insulin and clock started at completion of injection. Flush cannula with IV saline (10ml).
4. Samples drawn as follows:

08.00	- 60	0	2	3	4	6	9	12
	,	,	,	,	,	,	,	'
Glucose	x	x	x	x	x	x	x	x
GH		x		x	x	x	x	x
Cortisol	x	x		x	x	x	x	x
L.Hep (ml)	3	3	3	3	3	3	3	3
EDTA (ml)	5	7		5	7	5	5	5
Haemocue								
YSL								

Modified protocol for small children:

After 120' sample patient given breakfast but must remain resting on bed till after substantial meal. The cannula is removed when BSL levels are stable.

Supervision:

A doctor or Endocrine nurse must maintain continuous supervision throughout the test. A chart must be kept recording pulse and other symptoms eg: sweating, confusion, drowsiness etc. every 5 minutes from the start of the test. If untoward reaction occurs (coma, rapid palpitations, angina etc.) the test should be terminated with bolus of 50% glucose IV. (Adults ONLY). In children, large amounts of intravenous hyperosmolar glucose should be avoided, and hypoglycaemia treated with glucose 200 mg/kg IV over 3 minutes (10% dextrose, 2ml/kg over 3 minutes)⁵. If a test is terminated with IV glucose, hormone sampling should continue if the patient stabilises.

Ensure a good (carbohydrate) food intake "as breakfast" and later lunch once the test is completed.

Interpretation

Cortisol should rise above 500nmol/L.

Present normal cut off (as accepted for Health Department Childhood GH Advisory Committee) is 4µg/L (normal >7µg/L) (1µg/L = 2.7mU/L). Levels in childhood between 4 and 7µg/L suggest partial GH deficiency. The cut off is very arbitrary, but is the present local criterion. The same cut off applies to any physiologically or pharmacologically stimulated test.

The consensus criteria for absolute GH deficiency in adults is a stimulated GH <3µg/L.

⁵ see Shah et al, Brit Med J 304: 173 – 174, 1992

GH Suppression Tests

Glucose Infusion Test for Growth Hormone

Clinical Applications:

This test can be useful but clinical consultation with an Endocrinologist must precede the test being performed.

Purpose of Test:

To determine the suppressive effect of glucose on Growth Hormone in patients with suspected acromegaly.

Patient Preparation

Patient fasted and rested for at least 30 minutes. High carbohydrate diet not necessary. Weight of patient.

Equipment:

1. 3 x 50ml syringes
2. 5 x 10ml syringes
3. 4 x 1ml syringes
4. Heparin syringes
5. 1 x IV set
6. 2 x scalp vein needles
7. 5 x 7ml EDTA tubes
8. Timing clock
9. 9 x 3ml Heparin tubes
10. Ampoules of 50% Dextrose
11. Bags of 10% Glucose solution

Possible side effects:

Transient faintness during initial bolus of glucose. Diuresis during infusion.

Procedure:

The glucose infusion is carried out over 90 min.

- Insert needle into vein, take -30' samples for GH, (Insulin and Glucose).
- Draw 0 sample.
- Insert needle into large vein on other arm and inject 0.5g glucose/kg intravenously over 3 minutes, restarting clock at mid-point of infusion (ie: 1½ min). Usual dose is 25g unless patient is very large. Connect 10% glucose solution and infuse at a rate to obtain blood glucose level of 16 - 17 mmol/L.
- Blood samples taken at 30, 60 and 90 min after commencement of infusion for glucose, GH and insulin. Samples for glucose only taken at 15, 45 and 75 min. Adjust rate of infusion in order to attain glucose level of 16 - 17 mmol/L for 60 min if possible.

Interpretation

Normal people should show suppression of GH levels to less than 3µg/L with sustained glucose levels of 11 - 17 mmol/L. Acromegalics will not shut off GH secretion. Elevated values are generally seen throughout, or a paradoxical increase in GH may occur.

References:

Cerasi and Luft. Acta Endocr. 1967; 55: 278.

Glucose Suppression Test (oral)

Clinical applications:

Diagnosis of Acromegaly.

Patient preparation:

Patient needs to be fasted overnight, water only after 9 p.m.

Procedure:

Blood sample at time 0. (Also at -30' and -15 if for diagnosis of acromegaly).
75g oral glucose orally.

Blood sample at 30, 60, 90 and 120 minutes.
Samples assayed for glucose and GH

Interpretation

1. GH should suppress to <math><0.3\mu\text{g/L}</math> after glucose
Values greater than this suggest acromegaly.
2. Normal fasting glucose is <math><5.6\text{mmol/L}</math>.
$5.6 - 6.96\text{mmol/L}$ impaired fasting glycaemia.
$\geq 7.6\text{mmol/L}$ diabetes (on 2 occasions)
3. 2 hour glucose <math><7.86\text{mmol/L}</math> normal glucose tolerance.
$7.8-11.06\text{mmol/L}$ impaired glucose tolerance
$\geq 11.16\text{mmol/L}$ diabetes mellitus

Glucose Tolerance Test (intravenous)

Clinical Applications:

This test can be useful but clinical consultation with an Endocrinologist must precede the test being performed.

Patient Preparation

Patient to have approximately 300g of carbohydrate daily for 3 days before the test. Fast overnight after a good supper. Patient to be lying for 30 minutes before the start and throughout the test. Patients height and weight recorded and any medication given in the previous 2 days.

Equipment:

1. 1 x 50ml syringe (for glucose)
2. 8 x 5ml syringes
3. 2 x 1ml syringes
4. 1 x aspirating needle
5. 2 x 21G butterfly needles
6. 2 x three way stopcocks heparin syringes
7. Glucose solution - 50ml of 50% solution (25g of glucose) in 50ml syringe.
8. 10 x 3ml Heparin vacutainers (for glucose)
9. 8 x EDTA tubes for insulin (if required)
10. timing clock
11. ice

Note: Fill in insulin assay form, (if required).

Procedure:

1. Insert 21G butterfly needle, obtain sample, attach heparin syringe and inject small amount to keep line patent. Insert second butterfly needle into opposite arm and inject 50ml of 50% glucose (25g). Restart the timer at mid-point of the infusion. Commence taking blood sample 15 seconds before sample is due. This timing is important, note any variation. Note start and finish time of each sample in label eg: 4'45" - 5'00".
2. Samples as follows:
Insulin: 5ml EDTA 0' 3' 5' 10' 20' 40' 60' 90'
Glucose: 0' 3' 5' 10' 20' 30' 40' 50' 60' 90'
3. Collection of samples:
 - Samples drawn without tourniquet wherever possible.
 - Gently rotate tubes to dissolve anticoagulant.
 - Place EDTA into ice immediately and spin in cold room for 10 minutes at speed 7, pipette plasma into labelled tubes and freeze immediately. Insulin in the blood is rapidly destroyed at room temperature therefore storage and transport must be made deep frozen.
 - Glucose samples centrifuged and kept cold until tested.
 - All samples must be clearly labelled with permanent labels.

Gonadotrophin Releasing Hormone

Gonadotrophin releasing hormone is not measured in New Zealand.

However, there is a GnRH stimulation test in which LH and FSH are measured.. Refer to page 114.

Miller Test, (Water Deprivation Test) for Diabetes Insipidus

Clinical Applications:

Clinical consultation with an Endocrinologist must precede the test being performed.

Note: Many drugs may affect results. Check with Endocrinologist.

Preparation:

Fluid and food deprivation for 12 hours prior to test (exceptions will apply to severe cases). Record weight before start of deprivation and 0600 hours (0800 h in outpatients) day of test. Bladder emptied 0600 hrs (0800 h for outpatients) morning of test. Subject remains fluid and food deprived until test completed.

Procedure:

0800 hrs blood drawn for AVP, Na & K, Cr, glucose osmolality. Urine for osmolality. Weigh hourly until completion of test.

Urine collected hourly until osmolality constant (< 30m osmole per Kg variation). Draw another sample of blood for AVP, Na, K, Creatinine, glucose & osmolality before giving DDAVP into each nostril. (Total dose 20 µg intra-nasal).

Collect 1 further blood and urine osmolality specimen 1 hr after DDAVP has been given.

Interpretation:

Urine osmolality (mosmol/kg)*		Diagnosis
After fluid deprivation	After desmopression	
<300	>750	HDI
<300	<300	NDI
>750	>750	DDI, Normal
300-750	<750	partial HDI, or partial NDI, or DDI

Plasma osmolality should exceed 295 mosmol/kg.

*Reference range for urine osmolality in hospitalised patients without polyuria is 750-950 mOsm/kg.

HDI, Hypothalamic diabetes insipidus; NDI, nephrogenic diabetes insipidus; DDI, dipsogenic diabetes insipidus (primary polydipsia).

If results are equivocal, plotting the relationship of plasma AVP to concurrent plasma and urine osmolality on the following graphs is recommended. (Please note Endolab use a highly sensitive RIA for plasma AVP and these results are less applicable to commercial kit AVP assays).

References:

Miller M et al. Am. Intern. Med. 1970; 73: 721

Zerbe RL and Robertson GL. NEJM 1981; 305:1539-46.

Baylis P, Clin.Endo 1995; 43:507-510

Pentagastrin Screening Test for Calcitonin Provocation

Clinical Applications:

Clinical consultation with an Endocrinologist must precede the test being performed.

Purpose of Test:

To measure Calcitonin after pentagastrin infusion in patients suspect of having medullary thyroid carcinoma. This test is no longer used as a screening test in MEN2 kindred, with advent of genetic testing.

Patient Preparation

Fasting morning sample is preferred but not essential.

Blood samples:

Serum frozen as described. Collect 7ml blood sample in a chilled red-top venepuncture tube and allow blood to clot 1 - 4 hours at 4°C (ice bath or refrigerator). Centrifuge sample, preferably in a refrigerated centrifuge, and separate serum from cells. Do **NOT** use Lipaemic samples. Freeze serum immediately. Minimum volume of serum required for one estimation is 0.8ml but if dilutions are necessary more serum will be required.

Procedure:

Give: Pentagastrin dose 0.5 µg/kg given IV over 5 seconds.

Preparation of Pentagastrin:

The standard pentagastrin preparation is 250 µg/ml and can be diluted in 24ml of sterile saline to provide a solution with 10 µg/ml from which the appropriate dose is readily drawn and administered. The test is best performed with an indwelling needle and heparin lock.

Side Effects:

Patients should be warned to expect mild discomfort for the first minute or two after the injection with nausea, retro sternal discomfort and increased intestinal motility. These symptoms are short-lived and mild in degree.

Sampling:

1, 2 and 5 minutes after pentagastrin. The peak is at 1 - 2 minutes, and samples at 10 or 15 minutes are not required.

Normal Range:

The following table described the calcitonin levels of normal subjects upon stimulation studied at Nichols Institute (USA) after using the present assay:

Gender	Time	After Pentagastrin* Infusion	After Combined Calcium/Pentagastrin** Infusion
Female	1 min	2-26	6-82
	2 min	3-29	4-94
	5 min	3-23	5-76
Male	1 min	6-90	26-350
	2 min	10-84	32-350
	5 min	7-106	24-244

* 0.5 µg/kg/5 sec pentagastrin

** 2 mg calcium ion/kg infused at a constant rate for 1 minute, followed by 0.5 µg/kg/5 sec of pentagastrin.

Peripheral CRH test

Purpose of test

In patients with established cortisol excess (i.e. Cushing's Syndrome), the Peripheral CRH test is used to help differentiate Cushing's disease from ectopic ACTH production. This test should only be performed under the direction of an Endocrinologist with experience in the management of Cushing's Syndrome.

Protocol for peripheral ovine CRH test in differential diagnosis of ACTH dependent Cushing's syndrome.

Fluids only from 2400h.

Test to commence between 0900h and 1000h in an active phase of Cushing's syndrome (if cyclical) and clear of any adrenolytic therapy.

Sample times:

-15 min

-1 min

Give 1µg/kg oCRH over 1 minute IV following the -1 sample. Set time to 0' at completion of CRH.

15 min

30 min

45 min

60 min

90 min

Hormones to be measured ACTH and cortisol.

May be combined with IPSS at the discretion of the clinician ordering the test, with peripheral samples for cortisol and ACTH at above times (15 to 90 minutes) in addition to the IPSS protocol. There is no indication to perform further inferior petrosal sinus ACTH levels beyond 10 minutes.

Interpretation

An increase in ACTH concentration of >35% of the mean basal concentration at time 15 + 30 minutes distinguishes pituitary from ectopic ACTH with 100% specificity. Some patients with Cushing's disease will not demonstrate this degree of increase (sensitivity 93%). Cortisol should increase by >20% over mean basal concentration at time 30 + 45 minutes.

Reference

Nieman et al, 1993. JCEM 77: 1308-12

Short Metyrapone Test

Purpose of Test:

This test is designed to assess pituitary ACTH release. Metyrapone inhibits the last (11-hydroxylation) step in the synthesis of cortisol. The inhibitory effect of cortisol on ACTH secretion is thereby reduced and ACTH secretion is increased. The ACTH response can either be measured directly and indirectly by the increased secretion of plasma 11-deoxycortisol.

Indications:

Assessment of hypothalamic pituitary adrenal function especially:

Following pituitary surgery.

Equivocal short synacthen test.

Contraindications to insulin tolerance test.

Note: anticonvulsants may alter test results.

Procedure:

The test may be conducted as an inpatient or outpatient at the discretion of the Endocrinologist.

Where there is a high probability of adrenal insufficiency, important co-morbidities, compliance issues or social factors (such as transportation difficulties, geographic location), inpatient testing is recommended with overnight admission.

Patient should be euthyroid and not ingesting corticosteroids.

Give 30 mg/kg metyrapone between 2300h and 2400h with milk and a snack

2.0g for < 70 kg

2.5g for 70 - 90 kg

3.0g for >90 kg

Measure 0800 h cortisol, 11-deoxycortisol and ACTH.

Endocrine Tests Centre nurse to confirm compliance, enquire about side effects prior to drawing blood, and to check pulse and lying / standing blood pressure before discharge.

Side effects

In a large series from Ireland (Fiad et al. Clin Endocrinology 1999; 40: 603-609), side effects from the overnight metyrapone test using this dose occurred in only 7 out of 398 patients having 576 tests. Side effects included nausea and vomiting (3 patients), dizziness (2 patients), nightmares (2 patients) and one patient with unusual limb sensations and faintness.

Interpretation

Normal results:

11-deoxycortisol = >200nmol/L

ACTH = >20pmol/L

Cortisol = <200nmol/L (Indicates adequate suppression)

Secondary adrenal insufficiency (ACTH deficiency) if 11-deoxycortisol <200nmol/L and ACTH <20pmol/L in the presence of adequate suppression of plasma cortisol.

Possibility of partial ACTH deficiency where 11-deoxycortisol slightly >200nmol/L but failure of ACTH to rise.

Some authors suggest that ACTH should rise >30pmol/L (Steiner et al, Exp Clin Endocrinol, 1994; 102: 33-38).

Short Synacthen (Synthetic 1-24 ACTH) Test

Purpose of Test:

- To assess adrenal function in patients with suspected Addison's Disease, chronic ACTH deficiency or patients who have been receiving long term steroid therapy.
- Synacthen Stimulation Test can be done at any time of day, although 0800 - 1000 is preferred if possible.
- Samples for cortisol to be taken at time 0 (pre dose) and 30 minutes (post dose).

Patient Preparation

Patient need not be fasted and may be ambulant during the test. It is essential that the patient should not take cortisone, hydrocortisone, prednisone or prednisolone for at least 8 hours (and preferably 24 hours) prior to test. The patient's usual morning steroid dose may be taken immediately **after** the test is completed. This test is best done early am (eg. 0800h or 0900h), which provides extra information on basal plasma cortisol status. In emergency situations it is possible to do the stimulation test at any time. Please ensure sampling times are documented on the cortisol request form and blood tubes.

Procedure:

- Take blood for cortisol (pre-synacthen), and write the time on the tube (eg 0800h)
- Inject 0.25 mg synacthen IM (usually into deltoid) or IV via cannula.
- If giving synacthen IV, administer slowly over two minutes, do not dilute, and then flush the line with 5ml sodium chloride 0.9%.
- Before taking 30 minute post-synacthen blood, withdraw 2ml from IV line and discard.
- Take blood for cortisol 30 minutes post-synacthen. Label tube with time (eg. 0830h) and "post synacthen".

NB: Sometimes, synacthen tests are requested to diagnose late-onset congenital adrenal hyperplasia. The above protocol should be followed with blood samples taken for 17OH-progesterone at baseline (pre synacthen) and **60 minutes** after ACTH injection (post synacthen).

Interpretation

Post-synacthen cortisol of $>550\text{nmol/L}$ is normal, although if clinical suspicion remains high discussion with an Endocrinologist is recommended.

Post-synacthen cortisol of $<550\text{nmol/L}$ is abnormal. Endocrine review is recommended.

Patients with chronic ACTH deficiency may have normal responses. If high index of clinical suspicion suggest short metyrapone test (see page 112) or insulin tolerance test (see page 103).

TRH-TSH Releasing Hormone Stimulation Test/ GnRH Releasing Hormone Stimulation Test

Clinical Applications:

Clinical consultation with an Endocrinologist must precede the test being performed.

Note: GnRH is expensive and this test is rarely indicated on clinical grounds. An exception is in children with sexual precocity where an increased LH/FSH (of response to GnRH) is seen in central (true) sexual precocity. TRH testing has largely been replaced by sensitive plasma TSH assays for the diagnosis of thyrotoxicosis. TRH test is useful in diagnosis of TSHoma.

Purpose of Test:

Assessment of pituitary and target gland (thyroid and gonadal) function. This procedure is to be followed when both TRH and GnRH responses are needed. Previous work has shown that the response to one hormone is not altered by the addition of the other. It is often not necessary to carry out both these tests. Please specify which hormonal analyses are required.

Patient Preparation

Patient need not be fasting, but should empty bladder before the test.

Procedure:

Make up TRH 400 µg in diluent provided.

Make up GnRH 100 µg in diluent provided.

Inject TRH IV over one minute.

Then - Inject GnRH IV over one minute (set clock at "zero time" after TRH injection).

Note: either hormone may be given alone or can be given as part of an insulin tolerance test. Releasing hormone can be obtained by arrangement with the Christchurch Endocrinology Department.

Blood samples:

Should be taken at 0 (pre stimulation), 20, 60 and 90 min after injection. At each time the following is drawn:

TSH (TRH test)	5ml plain tube
LH (GnRH test)	} 5ml EDTA
FSH (GnRH test)	
Prolactin (TRH test)	

Separate plasma as soon as possible. Store plasma deep frozen. Label tube with hormone (LH etc) name, date and time of sample. All side effects should be noted. Date of last menstrual period, age, sex of patient, whether on oral contraceptives, thyroxine or other drugs should be noted and recorded. It is often valuable to know "end organ hormone" values at the time of testing (eg Free thyroxine index, plasma testosterone, etc).

Interpretation

Normal response.

TRH Stimulation:

TSH increment > 2.5 mIU/L (males)

> 5 mIU/L (females),

elderly males may have a minimal response.

Hyperthyroidism due to TSH secreting pituitary adenomas usually have reduced TSH response to TRH

Prolactin increment > 1050 mIU/L (more marked in females).

GnRH Stimulation

LH increment > 5 IU/l (more marked at midcycle or during the luteal phase).

FSH increment < 5 IU/l (more marked during puberty). (not always detectable).

Deficient responses are seen in TSHoma, hypopituitarism, thyrotoxicosis or patients receiving excess thyroxine (TSH and prolactin) or oestrogens (LH & FSH).

Exaggerated responses are seen in primary hypothyroidism (TSH and prolactin) and primary hypogonadism (FSH).

A paradoxical GH response to TRH (or GnRH) may be seen in acromegaly.


Patients with prolactinoma may fail to show a prolactin response to TRH.

In setting of raised T4 and normal or raised TSH, a deficient TSH response suggest TSHoma while a normal TSH response suggest syndromes of thyroid hormone resistance.

Appendices

Endolab Test Requisition Form

hormones - christchurch hospital - 0800 654465 - external 3640 848 (internal 80848)


endolab christchurch hospital

Surname		First names		Results/Copies to:		Endolab requisition N°		
D.O.B	Sex	Patient No.		Collected By:		Date:		
Ward	Hospital / Address		Consultant		Time		Despatch Time & Date	
Sanders reference number:		Charge to						

Adrenal	Fertility	Fluid Balance	Tumor/Miscellaneous	Pancreas	Pituitary
<input type="checkbox"/> 11-deoxycortisol <input type="checkbox"/> 17OH-progesterone <input type="checkbox"/> ACTH <input type="checkbox"/> Aldosterone (plasma) <input type="checkbox"/> Aldosterone (urine, 24hr) <input type="checkbox"/> CBG <input type="checkbox"/> Cortisol (plasma) <input type="checkbox"/> Cortisol (urine, 24hr) <input type="checkbox"/> CRH <input type="checkbox"/> DHEAS <input type="checkbox"/> Renin activity (PRA)	<input type="checkbox"/> Androstenedione <input type="checkbox"/> βHCG (serum) <input type="checkbox"/> βHCG (urine) <input type="checkbox"/> Dihydrotestosterone <input type="checkbox"/> FSH <input type="checkbox"/> Inhibin B <input type="checkbox"/> LH <input type="checkbox"/> Oestradiol 17β (sensitive) <input type="checkbox"/> Oestrone (urine) <input type="checkbox"/> Ovulation profile (urine) <input type="checkbox"/> Pregnenediol (urine) <input type="checkbox"/> Progesterone <input type="checkbox"/> Prolactin (PRL) <input type="checkbox"/> Testosterone (+SHBG)	<input type="checkbox"/> Aldosterone (plasma) <input type="checkbox"/> Aldosterone (urine, 24hr) <input type="checkbox"/> Angiotensin II (All) <input type="checkbox"/> AVP (vasopressin / ADH) <input type="checkbox"/> Catecholamines (plasma) <input type="checkbox"/> Renin activity (PRA)	<input type="checkbox"/> α-subunit <input type="checkbox"/> Calcitonin <input type="checkbox"/> PTH related peptide <input type="checkbox"/> 1,25 (OH) ₂ Vitamin D <input type="checkbox"/> Thyroglobulin	<input type="checkbox"/> C-Peptide <input type="checkbox"/> Insulin <input type="checkbox"/> Insulin antibodies <input type="checkbox"/> GAD <input type="checkbox"/> IA2 <input type="checkbox"/> ICA	<input type="checkbox"/> LH/FSH <input type="checkbox"/> IGF-1 <input type="checkbox"/> Prolactin <input type="checkbox"/> FPRL <input type="checkbox"/> Cortisol <input type="checkbox"/> T4/FTI
<input type="checkbox"/> T3 <input type="checkbox"/> T4/FTI <input type="checkbox"/> TSH <input type="checkbox"/> Thyroid antibodies <input type="checkbox"/> Thyroglobulin		<input type="checkbox"/> Gastrin <input type="checkbox"/> Glucagon <input type="checkbox"/> Pancreatic Polypeptide <input type="checkbox"/> VIP	<input type="checkbox"/> ACE <input type="checkbox"/> Bone ALP <input type="checkbox"/> C-Telopeptide <input type="checkbox"/> PTH (parathyroid hormone) <input type="checkbox"/> Vitamin D	Dynamic tests <input type="checkbox"/> Clonidine <input type="checkbox"/> Synacthen <input type="checkbox"/> Dexamethasone suppression (overnight) <input type="checkbox"/> Metyrapone suppression (overnight)	
Thyroid		24hr urines <input type="checkbox"/> Catecholamines + mets <input type="checkbox"/> Cortisol <input type="checkbox"/> Aldosterone <input type="checkbox"/> SHIAA	Bone / Calcium <input type="checkbox"/> ACE <input type="checkbox"/> Bone ALP <input type="checkbox"/> C-Telopeptide <input type="checkbox"/> PTH (parathyroid hormone) <input type="checkbox"/> Vitamin D	Growth <input type="checkbox"/> Growth hormone <input type="checkbox"/> IGF-1 <input type="checkbox"/> IGF-BP3	


Time of Multiple Sampling																			
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Clinical details

Other Tests:

Signature:

refer to endolab handbook or www.cdhb.govt.nz/chlabs for sampling & transport requirements



These request forms are available from:

Johanna Verdonk
 Endolab
 Christchurch Hospital
 Phone: (03) 364 0848
 Fax: (03) 364 0818
 Freephone: 0800-3636 552
 E-mail: johanna.verdonk@cdhb.govt.nz

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