

Adrenocorticotrophin (ACTH) (plasma)

Introduction

Adrenocorticotrophic hormone (ACTH) is secreted by the anterior pituitary corticotroph cells under the control of hypothalamic corticotrophin releasing hormone (CRH) to maintain the fascicular and reticular zones of the adrenal cortex and to stimulate the production of adrenal steroids. Hypothalamic secretion of CRH and release of pituitary ACTH are modulated by circulating cortisol in negative feedback loops. The secretion of ACTH occurs in a circadian rhythm with the lowest levels occurring shortly after sleep (midnight) and peak levels occurring shortly after waking (08.00-09.00). ACTH is a single chain polypeptide (MW = 4540 daltons) consisting of 39 amino acids, of which the first 24 are essential for its biological activity. Synacthen is a synthetic 1-24 analogue of ACTH, which is used in tests to stimulate the adrenal cortex. Like other small peptide hormones, ACTH has a short half-life of 5-10 minutes in plasma.

Clinical Indications

1. Establishment of the aetiology of Cushing's syndrome

ACTH estimations are used to establish aetiology in patients with **proven** Cushing's syndrome. For this purpose, ACTH is usually measured on single samples taken at 09.00 or on several samples during a dynamic test such as the CRH test.

2. Assessment of treatment of patients with Cushing's syndrome

ACTH estimations are used to predict the likelihood of development of Nelson's syndrome after bilateral adrenalectomy and assess the effectiveness of pituitary tumour treatment (pituitary irradiation, chemotherapy or surgery).

3. Differentiation of primary from secondary causes of adrenal insufficiency

In patients with proven adrenal insufficiency, measurement of ACTH in a 09.00h plasma sample permits distinction between pituitary and adrenal causes.

4. Localisation of excess ACTH production

Selective venous sampling is helpful in locating the source of ACTH in patients where imaging is equivocal.

Measurement of ACTH is rarely indicated in the diagnosis of Cushing's syndrome, congenital adrenal hyperplasia, hirsutes, delayed puberty, hypopituitarism or during an insulin-induced hypoglycaemia test.

Method Information

All centres offering this assay use a solid-phase, two-site sequential chemiluminescent immunometric assay specific for intact ACTH (1-39). These assays show <1% cross-reactivity with ACTH (1-18), ACTH (1-24) and alpha MSH. There is approximately 18% cross-reactivity with ACTH (18-39). It is important to note that these assays do not therefore detect Synacthen administration. The lowest reported level is 5 ng/L and intra- and inter-assay precision is <10% for ACTH values between 20 and 300 ng/L.

Patient Preparation

For Clinical Indications **1,2** and **3** (see above) there is no special patient preparation. Between 09.00h and 10.00h, take blood (4-5 mL) from a forearm vein following the guidance under 'Sample Requirements'.

Failure to obtain blood at the first attempt may invalidate the results of subsequent successful venepuncture. In particularly anxious patients, the ACTH concentration may rise to twice the upper limit of normal. In such patients sampling 30 min after the insertion of a butterfly is advised.

Where patients are receiving replacement therapy with corticosteroids, the sample should be taken immediately before the morning dose of steroid. The time of the previous dose **must** be recorded.

For Clinical Indication 4, (see above) selective venous sampling should be carried out by a radiologist and endocrinologist experienced in the technique. Catheters are inserted into both inferior petrosal veins, via the femoral vein. A cannula is also inserted into a peripheral vein. Protocols vary, but generally blood is taken simultaneously from the three sites before and 2, 5, 10 and 15 min after an intravenous injection of 100 mg of corticotrophin releasing hormone (CRH). Blood samples must be placed immediately in ice and, at the end of the procedure, taken to the laboratory for immediate separation. The plasma should be divided into two portions and stored at -20°C.

Sample Requirements

An EDTA plasma sample is required, transported to the laboratory 'on ice' and separated within 30 minutes of venepuncture. A siliconised glass EDTA vacutainer may be used or a plastic syringe/plastic EDTA tube. (Non-siliconised glass tubes bind ACTH and produce erroneously low results). The plasma should be divided into two portions and stored at -20°C.

Sample Handling

The whole blood, in either an EDTA siliconised glass vacutainer or a plastic EDTA conventional tube, must be transferred on ice to the laboratory within 30 minutes of sampling. Separate the plasma using a refrigerated centrifuge and transfer each plasma sample to two plastic tubes and immediately freeze at -20°C.

N.B. Falsely low ACTH results are caused by the presence of heparin. Samples in which this anti-coagulant has been used will be discarded.

Send one sample from the pair to the SAS laboratory. The minimum sample volume is 0.5 mL. Ensure the sample remains frozen during transport. Hold the remaining portion of the plasma frozen in reserve. Record on the SAS request form the time of day at which the sample was taken and the time and dose of any previous corticosteroid treatment.

Interpretation

Reference Ranges:

Time	Newcastle	St Bart's	St Thomas'
09.00	<47 ng/L	<50 ng/L	5 – 46 ng/L
Midnight	<10 ng/L	<10 ng/L	<10 ng/L

For interpretation of ACTH results during dynamic tests, consult the appropriate SAS centre.

Inferior petrosal sinus sampling after CRH stimulation.

A gradient of the ACTH concentration of more than two-fold between one petrosal sinus and peripheral samples indicates a pituitary adenoma secreting excess ACTH.

Quality Assessment

UK NEQAS.

Centres offering this assay

London (St. Bart's), London (St. Thomas'), Newcastle.

References

Landolt AM, Valavanis A, Girard J, Eberle AN. Corticotrophin-releasing factor-test used with bilateral, simultaneous inferior petrosal sinus blood-sampling for the diagnosis of pituitary-dependent Cushing's disease. *Clin Endocrinol* 1986; **25**: 687-696.

Perry LA, Grossman AB. The role of the laboratory in the diagnosis of Cushing's syndrome. *Ann Clin Biochem* 1997; **34**: 345-359.

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Aldosterone (plasma, serum)

Clinical Indications

Investigation of disorders of aldosterone production. Concomitant plasma renin activity (PRA) measurements are required for both initial diagnosis and differentiation between many of the conditions listed below.

These disorders are:

1. *Primary hyperaldosteronism.* Aldosterone secreting adenoma (Conn's syndrome) and related conditions.
2. *Secondary hyperaldosteronism.* Inherited renal tubulopathies. Bartter's syndrome, Gitelman's syndrome and pseudohypoaldosteronism are groups of disorders of impaired potassium, chloride and water reabsorption in the renal tubules with pronounced salt wasting.

In secondary hyperaldosteronism associated with nephrotic syndrome, cirrhosis or with renal hypertension aldosterone assays are not helpful, although the measurement of PRA may assist management (See Renin Activity).

3. *Primary hypoaldosteronism.* Isolated deficiency of aldosterone synthesis. Renin activity is stimulated when salt loss occurs due to hypoaldosteronism. The excess secretion of mineralocorticoids such as deoxycorticosterone suppresses renin production in some cases.
4. *Secondary hypoaldosteronism.* Hyporeninaemic hypoaldosteronism results from kidney damage and may be associated with diabetes mellitus or interstitial nephritis.

Low aldosterone with hypertension is found in certain renal tubulopathies

- Liddle's syndrome associated with hypokalaemia
- Gordon's syndrome with hyperkalaemia.

In Cushing's syndrome due to ectopic ACTH secretion and in states of glucocorticoid resistance renin activity is suppressed and aldosterone concentrations are low. Cortisol can act as a mineralocorticoid in Cushing's syndrome and the syndrome of apparent mineralocorticoid excess due to defect of 11 β -hydroxysteroid dehydrogenase type 2 that normally inactivates cortisol to cortisone in the kidney.

Hypertension in pregnancy can be very rarely due to progesterone acting on an aberrant mineralocorticoid receptor.

Applications

1. Primary hyperaldosteronism.

These conditions, where patients present with hypertension and in some cases sustained hypokalaemia, may be subdivided into:

- that due to a unilateral adenoma of the zona glomerulosa: Conn's syndrome.
- that due to bilateral nodular hyperplasia of the adrenal zona glomerulosa: idiopathic hyperaldosteronism
- that due to the expression of a chimeric gene of cortisol 11-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2): glucocorticoid-remediable hyperaldosteronism. (GRA).

2. *Secondary hyperaldosteronism.*

Inherited renal tubulopathies usually present in childhood and persists in varying severity in adulthood. Diuretic/laxative abuse and psychogenic vomiting should be excluded.

(a) Bartter's syndrome.

This recessive disorder is characterised by renal salt wasting and hypokalaemic metabolic alkalosis with normal calciuria or hypercalciuria. This syndrome results from mutations in one of several membrane transporter genes of the medullary thick ascending limb of renal tubules. The incidence is estimated to be 1.2 per million. Polyuria and polydipsia are invariably present in children and adults presenting with Bartter's syndrome. The majority present as neonates with a salt losing crisis. Profound salt loss leads to a compensatory increase in PRA and juxtaglomerular cell hyperplasia. The consequent increase in aldosterone levels exacerbates potassium loss and patients present with sustained hypokalaemia but without hypertension.

Affected genes are:

Type 1 Bartter's syndrome – mutations in sodium potassium 2 chloride co-transporter gene SLC12A1 that influences salt reabsorption, hypercalciuria and low blood pressure.

Type 2 Bartter's syndrome – mutations in potassium channel gene ROMK

Note: Type 1 and 2 are antenatal forms with polyuria in utero that leads to polyhydramnios.

Type 3 Bartter's syndrome is the classic form now attributed to mutations in basolateral chloride channel gene CLCNKB. Type 3 should be considered in children with growth retardation. The primary defect affects chloride reabsorption in the loop of Henle. There is renal potassium wasting and sometimes hypomagnesaemia.

Type 4 Bartter's syndrome – mutations in the barttin gene BSND. This is an autosomal recessive disorder with hypokalaemic, metabolic alkalosis.

The Gitelman variant is often asymptomatic into adult life, presenting with weakness, paraesthesia, tetany and severe fatigue. The molecular basis is mutation in the thiazide-sensitive sodium chloride co-transporter gene (SLC12A3) with hypokalaemic alkalosis in conjunction with hypocalciuria and hypomagnesaemia.

(b) Pseudohypoaldosteronism.

This condition may be suspected in hyperkalaemic, salt-losing infants who fail to thrive but in whom 21-hydroxylase deficiency has been excluded.

Type 1a is an autosomal recessive defect in the amiloride-sensitive epithelial sodium transport channel gene (ENaC). Plasma renin activity is grossly elevated by the reaction of the juxtaglomerular apparatus to profound salt loss. Aldosterone is elevated, giving an impression of end-organ insensitivity to aldosterone. ENaC function in lung epithelia may be impaired leading to recurrent chest infections and may be mistaken for cystic fibrosis.

Type 1b is an autosomal dominant form due to mutations in the mineralocorticoid receptor gene. Patients respond to a high sodium diet and the condition usually resolves.

3. *Primary hypoaldosteronism.*

Isolated primary hypoaldosteronism results from specific interference with aldosterone production, either through enzyme deficiency in aldosterone synthesis, or atrophy/ destruction of the zona glomerulosa. Initial investigations should exclude congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency (17-hydroxyprogesterone measurement or urine steroid profile) and Addison's disease. ACTH and cortisol should be measured.

4. *Secondary hypoaldosteronism.*

Hyporeninaemic hypoaldosteronism results from kidney damage and may be associated with diabetes mellitus or interstitial nephritis.

Liddle's syndrome is a rare hypertensive disorder with low plasma renin activity and aldosterone concentrations. The primary defect is a constitutive activation of the renal epithelial sodium channel due to mutations in either the beta subunit (SCNN1B) or the gamma subunit (SCNN1G) of this channel.

Gordon's syndrome (Pseudohypoaldosteronism type 2) presents as hyperkalaemic, hyperchloraemic acidosis in the neonatal period or hyperkalaemia with hypertension in children and adults.. The condition arises from mutations in the WNK4 gene.

Investigation of patients with hypertension.

The diagnosis of hyperaldosteronism should be considered in:

- hypertensive patients requiring 3 or more anti-hypertensive drugs,
- young adult hypertensives
- hypertensive patients found to have an adrenal mass
- hypertensive patients with hypokalaemia when on ACE inhibitors
- hypertensive patients with spontaneous or diuretic induced hypokalaemia especially in the presence of a sodium >140 mmol/L

Recent work suggests that up to 50 % of patients with primary aldosteronism are normokalaemic.

Patient Preparation.

Where practical all drugs should be discontinued for 2 weeks before samples are collected.

For first line screening tests, antihypertensive drugs may be continued with the exception of aldosterone antagonists (e.g. spironolactone) or oestrogens (stop for at least 6 weeks before the aldosterone-renin system is assessed) and β -blockers (stop for 2 weeks). The α -blockers, doxazosin and prazosin, have little effect on the aldosterone-renin system and they may be substituted. Hydralazine and slow release forms of verapamil have little effect on the ratio. Some β -Blockers depress renin activity more than aldosterone and give a false-positive ratio. Calcium channel blockers and ACE inhibitors may reduce the ratio. If the screening test is equivocal it should be repeated after stopping calcium channel blockers and ACE inhibitors for 2 weeks.

The patient must be receiving an adequate intake of sodium (100-150 mmol/day) and potassium (50-100 mmol/day). In patients with persistent hypokalaemia it is advisable to restore plasma potassium concentration to within the reference range or, if this is not attainable, to the maximum concentration possible. Discontinue this supplementation 24h before blood samples are taken.

***Out-patient screening procedure**

In many cases a diagnosis may be indicated from the measurement of aldosterone concentration and renin activity with calculation of an aldosterone to renin ratio measured in an out-patient clinic without the need for overnight hospital admission. A suppressed or low plasma renin activity with a high aldosterone/renin ratio is suggestive of primary aldosteronism in the absence of treatment with a β -blocker. Drug therapy, diurnal variation and patient posture have less affect on the ratio than the individual measurements. The ratio helps to diagnose those patients with hypertension in whom the renin is suppressed but aldosterone is normal or only marginally elevated.

Further tests will help to distinguish the aetiology of primary hyperaldosteronism: unilateral adenoma from bilateral hyperplasia.

After the patient has rested quietly for at least 10 min, take blood for measurement of aldosterone (5 mL) and PRA (5 mL, see Renin Activity) and electrolytes.

If primary aldosteronism is indicated by the aldosterone/renin ratio, confirmed when off all interfering drugs, then MRI or CT scanning should be advised to try to locate an adenoma.

The results of an aldosterone to renin ratio in this screening procedure may be equivocal and the patient will then require further investigation as an in-patient.

***In-patient posture procedure**

Postural studies can be used for distinction of adenoma and hyperplasia. In the latter there is a rise of aldosterone on standing because it is an Angiotensin II responsive condition, whereas with an adenoma there is a fall in aldosterone through the day because the majority are responsive to ACTH. Sensitivity is poor and in 50% of adenoma cases there is no decrease in aldosterone. Patients must observe strict overnight recumbency. After waking, the patient must remain lying down and must not alter posture in any way until after the initial blood sample has been taken.

Collect blood samples as follows:

08.00h: after overnight recumbency and before breakfast take blood for measurement of aldosterone and cortisol (5 mL) and PRA (5 mL, see Renin Activity).

08.30h: after the patient has been out of bed for 30 min and before breakfast take blood (5 mL) for PRA measurement.

12.00h: after the patient has been out of bed since the 08.00h sample and before lunch take blood (5mL) for plasma aldosterone and cortisol measurement.

The diagnosis of glucocorticoid remediable aldosteronism (GRA) is suspected when plasma aldosterone is high regardless of conventional anti-hypertensive treatment. High concentrations of 18-hydroxycortisol in blood or urine are characteristic. Aldosterone is suppressed and remains suppressed on long term treatment with dexamethasone.

Investigation of primary and secondary hypoaldosteronism.

A urine steroid profile analysis is useful for defining raised metabolites of intermediates in the synthesis of aldosterone (corticosterone, 18-hydroxycorticosterone) and cortisol (11-deoxycortisol, 17-hydroxyprogesterone).

A short Synacthen test is helpful in making a diagnosis of hypoaldosteronism. Take the baseline blood for the measurement of aldosterone and cortisol (5 mL) and PRA (5 mL, see Renin Activity). Give Synacthen (250 microgram i.m.) and take a further sample (5 mL) 30 min later for the measurement of aldosterone and cortisol.

Sample Preparation

Plasma:

Samples for the assay of aldosterone may be made on plasma or serum but since renin activity measurement is usually required, the preferred sample is plasma. Collect blood (5 mL) at room temperature, centrifuge as soon as possible after collection (maximum 2 hours), separate and freeze plasma rapidly. The plasma must remain frozen for storage and transport (see Renin Activity). Samples requiring the assay of aldosterone and PRA should be sent in two portions where possible to facilitate rapid turnaround time.

Send one unfrozen portion of the plasma to the local laboratory for measurement of plasma electrolytes. Send other portions (1 mL each) to the SAS laboratory. Store the remaining sample frozen until results of the assay are available.

A urine sample should also be collected for electrolyte analysis.

Record on the SAS request form the plasma electrolyte values, blood pressure and drug history.

Reference ranges

Please contact the appropriate SAS laboratory.

Adults (age 20 to 40 years)

(Sodium intake 100 - 150 mmol/day, potassium intake 50 - 100 mmol/day):

Plasma/serum: 08.00h after overnight recumbency: 100 - 500 pmol/L. Afro-Caribbeans have lower aldosterone and PRA. Plasma aldosterone usually exceeds 400 pmol/L in Conn's syndrome (unilateral adenoma).

In adults, baseline plasma/serum aldosterone concentrations and the increment in response to changing from a supine to an upright position decline with advancing age. According to some authorities, the mean values for both these indices, after 60 years of age, are about half those of young adults.

Infants

Reference ranges for plasma/serum aldosterone are much higher than in adults. In the first few weeks of life values of up to 5000 pmol/L have been reported. These high concentrations decline rapidly in the first year and then more slowly attaining, by 6 years, values similar to those of adults. Please check with the SAS laboratory that performed the assay.

These reference ranges should be considered with those quoted for PRA (- see Renin Activity).

Interpretation of Results

Electrolyte levels in plasma and urine and PRA values must be taken into account in the interpretation of aldosterone results (see Renin Activity).

Plasma:

1. Primary hyperaldosteronism.

Diagnosis of primary hyperaldosteronism traditionally rested on the finding of suppressed PRA with inappropriately elevated aldosterone levels in a patient who is hypertensive and hypokalaemic and in whom urine potassium levels show kaliuria inappropriate for the corresponding plasma potassium levels. Recent studies indicate that more emphasis is given to the aldosterone/renin ratio in patients with high blood pressure resistant to therapy in whom plasma potassium is not necessarily low.

Screening procedure – A suppressed plasma renin activity and high aldosterone/renin ratio is suggestive of primary aldosteronism. If the ratio of aldosterone (in pmol/L) to PRA (in pmol/mL/h) is 2000 or more, primary hyperaldosteronism is very likely, if >1,000 it is possible and <800 it is unlikely.

*In-patient procedure:

Primary hyperaldosteronism is indicated by an elevated aldosterone/renin ratio at 08.00h. A low renin activity that shows little or no increase after 30 min of mobility also supports autonomous aldosterone secretion. To aid the distinction between hyperaldosteronism due to adrenal adenoma and that due to bilateral adrenal hyperplasia, it may be helpful to consider the plasma aldosterone concentrations at 08.00h and 12.00h. In normal subjects and patients with adrenal hyperplasia, the aldosterone rises on standing. If cortisol values between 08.00h and 12.00h show a decrease due to normal diurnal rhythm, an elevated aldosterone level at 08.00h decreasing by 50% or more at 12.00h is suggestive of an ACTH sensitive condition such as an adenoma or GRA.

If primary hyperaldosteronism is indicated, CT or MRI scanning should be advised to try to locate an adenoma but beware: non-secreting incidentalomas are common.

If biochemical or imaging procedures are equivocal, there are further investigations that have been used to demonstrate primary hyperaldosteronism including the fludrocortisone suppression test, scintigraphy, salt-loading and other dynamic tests, but the patient should be referred to an endocrinologist with experience in these procedures.

If surgical removal of an adenoma is considered, confirmation of the source of excess aldosterone secretion should be obtained by adrenal vein catheterisation. This should be undertaken only in a specialist centre with a radiologist experienced in this difficult procedure. Samples should be taken from the low I.V.C., the high I.V.C., the left and right adrenal veins and a peripheral vein. It is advisable to confirm a high aldosterone/renin ratio in the peripheral sample at this time. The SAS laboratory will measure cortisol to facilitate interpretation of the results. There will be no charge for the additional assays.

GRA is a familial condition suspected when plasma aldosterone is not suppressed with saline infusion and when furosemide or a low salt diet does not stimulate plasma renin activity. 18-hydroxycortisol is a useful marker for GRA – this can be seen to be raised in a urine steroid profile or in plasma.

2. *Secondary aldosteronism.*

Bartter's syndrome.

Both the plasma concentration of aldosterone and the PRA at 08.00h after overnight recumbency are raised. In such patients who are normotensive, Bartter's syndrome should be suspected provided diuretic/laxative abuse and psychogenic vomiting have been excluded.

Pseudohypoaldosteronism.

The autosomal dominant form, usually found in children, is associated with a high plasma aldosterone concentration and very high PRA in the face of salt loss. The patient responds to high sodium intake and the condition eventually resolves. The autosomal recessive form persists into adulthood. Amiloride is the treatment of choice.

3. *Primary hypoaldosteronism.*

Failure of aldosterone concentrations to increase by at least 150 pmol/L from baseline 30 min after administration of Synacthen (250 microgram i.m.) is indicative of inadequate zona glomerulosa function. A diagnosis of isolated primary hypoaldosteronism rests upon raised PRA, low plasma aldosterone concentration, inadequate response of aldosterone to ACTH associated with a normal cortisol response.

4. *Secondary hypoaldosteronism.*

In this situation, both plasma aldosterone concentration and PRA are subnormal.

Quality Assessment

UK NEQAS, BioRad and sample exchange between centres.

Centres offering this assay.

Leeds, London (St Mary's) and London (UCLH)

References

Cartledge S, Lawson N. Aldosterone and renin measurements. *Ann Clin Biochem* 2000; **37**: 262-78.

Nadar S, Lip GYH, Bevers DG. Primary hyperaldosteronism. *Ann Clin Biochem* 2003; **40**: 439-52.

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Sayer JA, Pearce SH. Diagnosis and clinical biochemistry of inherited tubulopathies. *Ann Clin Biochem* 2001; **38**: 459-70.

Young WF. Minireview: Primary Aldosteronism- Changing Concepts in Diagnosis and Treatment. *Endocrinology* 2003; **144**: 2208-13.

Alpha Subunit (ASU) (serum)

Introduction

The alpha subunit of the pituitary glycoprotein hormones (TSH, LH and FSH) has a common structure and is non-covalently bound to a beta subunit with the latter being hormone specific. ASU is also secreted as a free molecule and hypersecretion has been reported in patients with various pituitary tumours. It may also be elevated in primary hypogonadism, renal failure and primary thyroid disease.

Clinical Indications

1. Detection and monitoring of functionless pituitary tumours.
There is some evidence that functionless pituitary tumours secrete increased quantities of the alpha subunit common to pituitary glycoprotein hormones.
2. Detection and monitoring of glycoprotein hormone secreting adenomas e.g. TSH, LH/FSH.
3. Detection and monitoring of malignant gastroenteropancreatic tumours.
4. Differential diagnosis of thyroid hormone resistance and TSH secreting adenomas.

Method Information

Birmingham

An in-house radioimmunoassay calibrated against NIBSC 75/569

St Barts' (London)

An in-house radioimmunoassay calibrated against NIBSC 75/569

For both assays there is a 0.1% cross-reactivity with hCG which may affect measured serum concentrations in pregnancy.

Patient Preparation

None. Take blood (5 mL) using a plain vacutainer or syringe.

Sample Handling

Transfer the blood to a plain tube and allow to clot. Clot accelerators and gel tubes should not be used. Separate the serum and store at -20°C if not for immediate despatch. Send serum (1 mL) to the SAS laboratory by First Class Post (Do not send on a Friday). Samples do not need to be sent frozen. Comprehensive clinical and laboratory information should accompany each request.

Interpretation

Please contact the SAS laboratory.

Raised values for the common alpha subunit are also found after the menopause, in renal failure, hypothyroidism, Cushing's syndrome, pregnancy and in some patients with malignancies of breast or pancreas.

Quality Assessment

No external quality assessment scheme is available. Regular sample and standard exchange between the two laboratories takes place to ensure the quality of results.

Centres offering this assay

Birmingham, St Bart's (London)

References

Grossman M, Trautman ME, Poer HS et al. Alpha_subunit and human chorionic gonadotropin-beta immunoreactivity in patients with malignant endocrine gastroenteropancreatic tumours. *Eur J Clin Invest* 1994; **24**: 131-136.

Samuels MH, Ridgway EC. Glycoprotein-secreting pituitary adenomas. *Baillière's Clin Endo Metab* 1995; **9**: 337-358.

Nobels FR et al Chromogranin A as a serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *J Clin Endocrinol Metab* 1997; **82**: 2622-8.

Brucker- Davis F et al. Thyrotropin-secreting pituitary tumors: diagnostic criteria, thyroid hormone sensitivity, and treatment outcomes in 25 patients followed at the National Institute of Health. *J Clin Endocrinol Metab* 1999; **84**: 476-86.

Samejima N et al serum alpha-subunit levels in patients with pituitary adenomas. *Clin Endocrinol* 2001; **54**: 479-84.

Androstenedione (serum)

Clinical Indications

1. Management of congenital adrenal hyperplasia due to 11 β - or 21-hydroxylase deficiency.
2. Diagnosis of 17 β -hydroxysteroid dehydrogenase deficiency.

Applications

1. Congenital adrenal hyperplasia (CAH).

Although androstenedione concentrations are increased in CAH due to an 11 β - or 21-hydroxylase deficiency, the steroids 11-deoxycortisol and 17 α -hydroxyprogesterone, respectively, are better diagnostic hormones. The measurement of androstenedione is particularly helpful in the management of such patients since suppression of the diagnostic steroids into the normal range is usually associated with symptoms of glucocorticoid excess. In addition, it is the androgens in particular which must be controlled to avoid virilisation.

2. 17 β -Hydroxysteroid dehydrogenase deficiency.

Male (46 XY) babies born with this enzyme deficiency have female or ambiguous genitalia. At puberty, marked virilization occurs. Early diagnosis and selection of gender for rearing are important. The condition is characterised by an increased serum concentration of androstenedione relative to that of testosterone. In infants, where androgen levels are normally low, androstenedione and testosterone should be measured after stimulation with human chorionic gonadotrophin (hCG).

Patient Preparation

1. Diagnosis and monitoring of congenital adrenal hyperplasia.

No patient preparation required. Take blood (2 mL). It is advisable to avoid stress.

2. Diagnosis of 17 β -Hydroxysteroid dehydrogenase deficiency.

For postpubertal children and adults, no patient preparation required. Take blood (2 mL).

For infants, stimulation with hCG is usually required to achieve serum androgen concentrations which can be measured with the precision necessary for accurate diagnosis.

Test combined with hCG stimulation*:

Day 1, 09.00h: Take blood (2 mL) into a plain tube for (baseline) androstenedione and testosterone assays. Give hCG, (i.m., 1500 IU for infants, 5000 IU for adults);

Day 4, 09.00h: Take blood (2 mL) into a plain tube for androstenedione and testosterone assays.

*Different protocols are used by different Centres. It is important to use the protocol of the Centre to which you send your samples, so that the Centre can interpret the results. Therefore contact the SAS Centre before carrying out this test.

Sample Preparation

Send serum samples (1 mL) to the SAS laboratory. Record on the SAS request form the time of sampling, details of any recent steroid therapy and, where appropriate, the time of hCG injection.

Reference Ranges

Adult males: 2-10 nmol/L;

Adult females: 3-12 nmol/L;

Prepubertal children: <3.5 nmol/L.

Androstenedione/testosterone ratio:

Unaffected males, all ages: <1.0;

Affected adults: >3.0;

Affected prepubertal children after hCG: >2.0.

Of particular diagnostic importance in 17 β -Hydroxysteroid dehydrogenase deficiency is an exaggerated rise in serum androstenedione concentration relative to the small increase in testosterone concentration in response to hCG. This is in marked contrast to the response in boys without the deficiency in whom testosterone concentrations increase much more than those of androstenedione.

In patients where 17 β -Hydroxysteroid dehydrogenase deficiency is suspected the SAS laboratory will undertake assay of both testosterone and androstenedione in order to advise on the interpretation of the androstenedione/testosterone ratio.

Quality Assessment

UK NEQAS.

Centres offering this assay

Leeds, London (St. Thomas').

References

Korth-Schutz et al. Serum androgens as a continuing index of adequacy of treatment of congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 1978; **46**: 452-458.

Rosler A. Steroid 17 β -Hydroxysteroid dehydrogenase deficiency in man: an inherited form of male pseudohermaphroditism. *J Steroid Biochem Molec Biol* 1992; **43**: 989-1002.

Arginine Vasopressin (AVP) / Anti-Diuretic Hormone (ADH) (plasma)

Clinical Indications

In the diagnosis of Diabetes Insipidus (DI) secondary to absolute or relative AVP deficiency or AVP resistance; in conjunction with clinical and other biochemical data.

High quality osmometry of plasma and urine samples obtained during strictly controlled water deprivation and after DDAVP administration is the most commonly used means of diagnosing DI and differentiating cranial and nephrogenic forms of the disease. This indirect approach can produce indeterminate results. Direct measurement of plasma AVP in response to graded osmolar stimulation is a highly accurate and reliable alternative approach, especially in those cases in which standard approaches have not been conclusive or in which additional defects in thirst perception are suspected (adipsic DI).

Patient Preparation

Drug (hypnotics, diuretics, opiates) therapy and alcohol ingestion must be discontinued if possible.

In the investigation of DI, plasma AVP measurements are only of value in association with the Hypertonic Saline Infusion Test which is a specialist procedure. A detailed protocol for this procedure and appropriate advice can be obtained from the SAS centre offering the AVP service.

Sample Preparation

Transfer the blood (minimum 5 mL, preferably 10 mL) to a plastic lithium heparin tube. Plasma must be separated within 30 minutes at 4°C and stored deep-frozen. Send samples deep-frozen to the SAS laboratory, ensuring that samples remain frozen during transport. If plasma osmolalities are measured in the local laboratory it should be stressed that high quality plasma results are required and the osmolality results should be written on the SAS request form*.

*N.B. The Newcastle SAS centre can provide a bespoke request form for AVP requests and a protocol for the Saline Infusion Test on request.

Reference Range

Interpretation is provided with the reported results.

Quality Assessment

No external quality assessment scheme exists for this assay and exchange of specimens has not to date been possible. Strict internal quality control procedures are followed, including the analysis of test samples in triplicate, with firm acceptance criteria.

Centre offering this assay

Newcastle.

Chromogranins A and B (plasma)

Clinical Indications

Diagnosis of neuroendocrine tumours and possibly pheochromocytomas. The chromogranins (CGA and CGB) are frequently found to be secreted in addition to the other gut hormones (VIP, Pancreatic polypeptide, Gastrin, Glucagon, Somatostatin and Neurotensin) in neuroendocrine tumours.

Patient Preparation

With the patient at rest after an overnight fast, take blood (10mL) into a Lithium Heparin vacutainer or, use a syringe and needle and add the blood to a Lithium Heparin tube (if the sample is part of a gut hormone screen) or plain tube for serum.

Sample Preparation

Immediately add Trasylol (aprotonin, 0.2mL, 2000 kIU/mL blood). Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result. Send at least 1mL plasma to the SAS laboratory each for CGA and CGB analysis. If the request is part of a full gut hormone screen an absolute minimum of 3mL of plasma must be sent. Ensure the sample remains frozen during transport.

CGA and CGB may be measured in serum which has been separated rapidly, frozen and transported frozen HOWEVER if the request is part of a GUT HORMONE screen or other components of the Gut Hormone screen may be required at a later date then the sample MUST be taken and prepared as described in the previous paragraph.

Reference Ranges

CGA	Adults (fasting)	<60 pmol/L
CGB	Adults (fasting)	>160 pmol/L

Quality Assessment

There are no external quality assessment schemes and there are no other centres offering this assay for sample exchange.

Centre offering this assay

London (Hammersmith).

References

Bishop AE, Bretherton-Watt D, Hamid QA, Fahey M, Shepherd N, Valentino K, Tatemoto K, Ghatei MA, Bloom SR, Polak JR. The occurrence of pancreastatin in tumours of the diffuse neuroendocrine system. *Mol Cell Probes* 1988; **2**: 225-35.

Sekiya K, Ghatei MA, Salahuddin MJ, Bishop AE, Hamid QA, Ibayashi H, Polak JM Bloom SR. Production of GAWK (chromogranin-B 420-493)-like immunoreactivity by endocrine tumors and its possible diagnostic value. *J Clin Invest* 1989; **83**: 1834-42.

Calcitonin
(plasma)

C-peptide (plasma, serum)

Clinical Indications

1. Differential diagnosis of documented spontaneous hypoglycaemia.
2. Evaluation of pancreatic β -cell function.

The measurement of both insulin and C-peptide is advisable in the investigation of hypoglycaemia since in some cases of insulinoma, insulin levels may be low or marginally inappropriate whereas C-peptide is almost always unequivocally raised. Both measurements are essential for the identification of factitious insulin administration. Further tests, such as those for ketones, proinsulin, sulphonylureas, growth hormone and the insulin-like growth factors may also be required to complete the diagnostic process.

C-peptide analysis may also be undertaken to evaluate pancreatic β -cell function and attempt to differentiate type I and II diabetes mellitus.

Patient Preparation

Hypoglycaemia, spontaneous or whilst fasting, must first be established by regular monitoring of blood glucose levels. Blood (10 mL) collected in a plain vacutainer or syringe, when the whole blood glucose concentration (confirmed by laboratory analysis) is less than 2.2 mmol/L (or less than 2.5 mmol/L in patients over 60 years), should be used for the tests. Vacutainers (7 mL, red top) may be used for blood collection. Small blood samples from neonates should be transferred to a heparin tube so that the maximum volume of plasma may be collected. A minimum volume of 0.2 mL should be stored at -20°C .

For the evaluation of pancreatic β -cell function, patients should not be receiving exogenous insulin therapy as this will suppress endogenous C-peptide production.

Sample Preparation

Transfer the blood to a plain tube. Separate the serum preferably within 30 min of collection and freeze at -20°C . Larger volumes of sample (5 mL) are required for additional studies such as IGF-1 measurements or sulphonylurea identification. Visible haemolysis may invalidate the result.

Send samples to the SAS laboratory. Ensure they remain frozen during transport. Record the blood glucose concentration on the request form.

Reference Range

An interpretation of the result(s) will be provided where sufficient information accompanies the request. For further clarification please contact the SAS Centre.

Quality Assessment

UK NEQAS.

Centres offering this assay

Cardiff, Guildford, London (Hammersmith).

Reference

Clark PM. Assays for insulin, proinsulin(s) and C-peptide. *Ann Clin Biochem* 1999; **36**: 541-564.

Marks V, Teale JD. Hypoglycaemia in the adult. In: Hypoglycaemia. Gregory JW, Aynsley-Green A (eds). Baillière's Clinical Endocrinology and Metabolism 1999; **7**: 705-729.

Marks V, Teale JD. Investigation of hypoglycaemia. *Clin Endocrinol* 1996; **44**: 133-136.

Ehtisman S, Barrett TG. The emergence of type 2 diabetes in childhood. *Ann Clin Biochem* 2004; **41**: 10-16.

Dehydroepiandrosterone Sulphate (serum)

Clinical Indications

1. Diagnosis of a virilizing adrenal tumour and monitoring recurrence after surgical removal.
2. Differential diagnosis of Cushing's syndrome due to an adrenal adenoma.

Applications

The assay has little value in the investigation of acne, idiopathic hirsutism or infertility.

1. Virilizing adrenal tumours.

These are often associated with an increased production of dehydroepiandrosterone sulphate which can be detected by analysis of serum or urine. Measurement is indicated in women if hirsutism and/or virilization is of sudden onset and in prepubertal children when pubic/axillary hair appears prematurely or there are other features of masculinization.

2. Cushing's syndrome.

Serum values below the normal reference range have been described in patients with cortisol excess due to adrenal adenoma.

Patient Preparation

No patient preparation required. Take blood (5 mL) into a plain tube. After surgery, samples should be taken as soon as practicable and at least every 2 months thereafter to monitor for recurrence.

Sample Preparation

Send serum (2 mL) to the SAS laboratory.

Reference Ranges

Serum: (Age-related reference ranges for adults are available from each Centre)

Pre-adrenarche: <0.5 mmol/L;

Adult males: 4-13 mmol/L (falling to <6 mmol/L by age 70);

Adult females: 3-11 mmol/L (falling to <5 mmol/L by age 70).

Quality Assessment

UK NEQAS.

Centres offering this assay

Leeds, London (St. Thomas').

11-Deoxycortisol (serum)

Clinical Indications

1. Diagnosis of, and monitoring therapeutic response in, congenital adrenal hyperplasia due to 11 β -hydroxylase deficiency.
2. Assessment of adrenal response in the metyrapone test.

Applications

1. Congenital adrenal hyperplasia (CAH).

11-Deoxycortisol, the immediate precursor of cortisol, accumulates in the peripheral circulation when there is a deficiency of adrenal 11 β -hydroxylase activity. The assay should be requested when CAH is suspected on clinical grounds, but serum 17 α -hydroxyprogesterone values are equivocal (14 - 100 nmol/L).

2. Long metyrapone test.

In the differential diagnosis of Cushing's syndrome, metyrapone is given to inhibit 11 β -hydroxylation. Subsequent changes in serum concentrations of 11-deoxycortisol and cortisol provide strong (but not absolute) indicators of the site of the primary lesion.

3. Overnight metyrapone test.

The 11-deoxycortisol response to the metyrapone-induced decrease in cortisol, provides an assessment of pituitary response.

Patient Preparation

1. Diagnosis of congenital adrenal hyperplasia.

Take blood (5 mL) at 09.00h.

2. Long metyrapone test.

The drug (750 mg orally 6-hourly for 2 days) is usually given combined with a snack to minimise epigastric discomfort. Glucocorticoid insufficiency may be precipitated, especially in patients with adrenal tumours. Take blood (5 mL) before administration of the first metyrapone dose (baseline sample) and 48h later for the measurement of 11-deoxycortisol and cortisol.

3. Overnight metyrapone test.

Metyrapone (30 mg/kg) is given orally with a snack at midnight. At 09.00h the next morning take 5 mL blood for the measurement of 11-deoxycortisol and cortisol.

Sample Preparation

1. Diagnosis of congenital adrenal hyperplasia.

Send serum (2 mL) to the SAS laboratory.

2. Long metyrapone test.

Send a portion (0.5 mL) of each serum sample to the local laboratory for measurement of cortisol. Send the remaining sample to the SAS laboratory. Record on the SAS request form the time of sampling and the serum cortisol concentration.

3. Overnight metyrapone test.

Send a portion (0.5 mL) of the serum sample to the local laboratory for the measurement of cortisol. Send the remaining sample to the SAS laboratory. Record on the SAS request form the time of sampling and the serum cortisol concentration.

Reference Ranges

1. *Diagnosis of 11 β -hydroxylase deficiency.*

Unaffected adults (09.00h): 5 - 12 nmol/L;
Unaffected neonates (>48h after birth): <20 nmol/L;
Patients with untreated 11 β -hydroxylase deficiency: >100 nmol/L.

2. *Long metyrapone test.*

- (a) Serum 11-deoxycortisol concentration >300 nmol/L at 48h, associated with a normal serum cortisol concentration, is strongly suggestive of Cushing's disease (pituitary lesion).
- (b) Serum 11-deoxycortisol concentration <150 nmol/L at 48h, associated with a subnormal cortisol value, suggests an adrenal adenoma.
- (c) Serum 11-deoxycortisol concentration >300 nmol/L at 48h, associated with subnormal or normal serum cortisol concentration, suggests that an ectopic source of ACTH exists. There is an overlap in the results of this test with those from patients with Cushing's disease.

3. *Overnight metyrapone test.*

A normal response of 11-deoxycortisol is regarded as >200 nmol/L. For the test to be valid the corresponding cortisol concentration should be <200 nmol/L. A subnormal response is suggestive of inadequate pituitary/ adrenal function.

Centre offering this assay

London (St Thomas²).

References

Fiad TM, Kirby JM, Cunningham S, McKenna TJ. The overnight single dose metyrapone test is a simple and reliable index of the hypothalamic-pituitary-adrenal axis. *Clin Endocrinol* 1994; **40**: 603-9.

18-hydroxycortisol (plasma, serum and urine)

Clinical Indication

As a second-line test in the investigation of primary hyperaldosteronism, to help distinguish between bilateral adrenal hyperplasia (normal levels) and Conn's adenoma (raised levels) or glucocorticoid - suppressible hyperaldosteronism (raised levels).

Application

Primary hyperaldosteronism can result from three main causes; bilateral adrenal hyperplasia, adrenal adenoma ("Conn's" adenoma), or glucocorticoid - suppressible (remediable) hyperaldosteronism ("GSH" or "GRH"), also known as "familial hyperaldosteronism type 1".

18- hydroxycortisol is a "hybrid" steroid produced by the action of the enzyme aldosterone synthase (CYP11B2), normally confined to the adrenal zona glomerulosa, on cortisol rather than the usual precursor corticosterone.

In bilateral adrenal hyperplasia, the normal architecture of the adrenal zones is maintained and plasma and urine 18-hydroxycortisol levels are normal.

Conn's adenomas often contain a mixture of cell types which allows cortisol to be converted to 18-hydroxycortisol. Some posture – responsive (Angiotensin II –responsive) adenomas contain a greater proportion of zona fasciculata-like cells and may not produce increased amounts of 18-hydroxycortisol¹.

GSH is a rare disorder caused by a hybrid gene consisting of the promoter region of the 11-hydroxylase gene (CYP11B1: ACTH responsive) and the functional region of aldosterone synthase (CYP11B2). The mutant enzyme produced is present in both the zona glomerulosa and zona fasciculata; it has 18-hydroxylase and 18-oxidase activity but is ACTH – dependant. Cortisol is available for conversion to 18-hydroxycortisol and so levels are high in plasma and urine.

Plasma and urine 18-hydroxycortisol determinations help to distinguish Conn's adenoma and GSH from bilateral adrenal hyperplasia. Some cases of posture-responsive Conn's adenoma may not have elevated levels. Urine samples analysed with the Southampton assay have been shown to give good discrimination of Conn's adenoma and GSH² and the diagnostic value of plasma compared to urine assays is being assessed.

Patient Preparation

Supine and / or ambulant plasma samples are suitable. No patient preparation is required.

Sample Preparation

24 hour urine sample

A 3-5 mL aliquot of a 24 hour urine sample with details of the 24 hour urine volume is required. Samples can be sent to the SAS laboratory by first class post.

Plasma or serum samples

Supine or ambulant EDTA or heparinised plasma or plain serum is suitable.

Samples can be sent in the first class post.

If renin/ aldosterone is to be measured as well then an EDTA plasma sample, frozen within 30 minutes of collection is required. Frozen transport is required for the renin assay.

Reference Ranges

Urine 18-hydroxycortisol

Adults - Men and women over 20 years of age 40 – 550 nmol/ 24 hours

Children and young adults (< 20 years) 5 – 820 nmol/ 24 hours

Plasma/ serum 18 – hydroxycortisol

Supine 0.7 - 6.5 nmol/L

Ambulant 1.6 – 10.7 nmol/L

Quality Assessment

No external quality assessment scheme is available.

Centre offering this assay

Southampton.

References

1. Stowasser M and Gordon RD. “ Primary aldosteronism.” In Best Practice and Research – Clinical Endocrinology and Metabolism (Ed Ferrari P) Elsevier Press 2003; **17 (4)**: 591-605.
2. Reynolds RM, Shakerdi LA, Sandhu K , Wallace AM, Wood PJ, Walker BR. The utility of three different methods for measuring urinary 18-hydroxycortisol in the differential diagnosis of suspected primary hyperaldosteronism. *Eur. J. Endocrinol.* 2005; **152 (6)**: 903-7.

Dexamethasone (serum)

Clinical Indications

1. Assessment of compliance in the overnight dexamethasone suppression test.
2. Assessment of the serum concentration of dexamethasone during corticosteroid replacement therapy.

Applications

1. Compliance.

Dexamethasone is administered for the diagnosis of Cushing's syndrome. Inadequate suppression of the cortisol concentration in serum may be due to non-compliance, Cushing's disease, endogenous depression or obesity. Non-compliance can be verified or dismissed by measuring the concentration of dexamethasone in serum.

2. Monitoring therapy.

The dose of dexamethasone (plasma half-life approx. 3 - 4h) required for adequate replacement varies widely between patients. Therapy may be monitored and regulated by use of this assay.

Patient Preparation

1. Compliance.

The patient is given a 1 mg dexamethasone tablet to be taken between 11 pm and midnight. Blood is taken for cortisol measurement at 09.00h.

In those patients who fail to suppress their cortisol levels after an overnight dexamethasone test, and in whom non-compliance is suspected, send 0.5 mL serum to the SAS laboratory. Record the cortisol result on the request form.

2. Monitoring replacement therapy.

Take blood (5 mL) into a plain tube immediately before and midway between doses of dexamethasone.

Sample Preparation

Send serum (0.5 mL) to the SAS laboratory. Record on the SAS request form the time of sampling, the serum cortisol concentration and (when monitoring therapy) the timing and dose of dexamethasone.

Reference Range

Compliance (1 mg dose): 2-16 nmol/L.

Centre offering this assay

London (St.Thomas').

References

English J, Chakraborty J, Marks V, Parke A. A radioimmunoassay procedure for dexamethasone. *Eur J Clin Pharmacol* 1975; **9**: 239-244.

Tsigos C and Chrousos GP. Differential diagnosis and management of Cushing's syndrome. *Ann Rev Med* 1996; **47**: 443-461.

Young MC, Cook N, Read GF and Hughes IA. The pharmacokinetics of low-dose dexamethasone in congenital adrenal hyperplasia. *Eur J Clin Pharmacol* 1989; **37**: 75-77.

5 α -Dihydrotestosterone (serum)

Clinical Indications

Diagnosis of 5 α -reductase deficiency.

Patients presenting with 46 XY karyotype and female or ambiguous genitalia may lack the enzyme testosterone 5 α -reductase. This enzyme activity is required for full masculinization of the external genitalia during intrauterine life. At puberty, virilization of affected subjects (often brought up as girls) may occur. 5 α -Reductase deficiency can be diagnosed after puberty by measurement of testosterone and 5 α -dihydrotestosterone (5 α -DHT) concentrations in the same serum sample. Before puberty stimulation of androgen production with human chorionic gonadotrophin (hCG) is usually required to achieve androgen concentrations which can be measured with the precision necessary for accurate diagnosis.

Patient Preparation

Test combined with hCG stimulation*:

Day 1 09.00h: Take blood (5 mL) into plain tube for (baseline) testosterone and 5 α -DHT assay. Give hCG (i.m., 1500 IU for infants; 5000 IU for adults);

Day 4 09.00h: Take blood (5 mL) into a plain tube for testosterone and 5 α -DHT measurements.

* Different protocols are used by different Centres. It is important to use the protocol of the Centre to which you send your samples, so that the Centre can interpret the results. Therefore contact the SAS Centre before carrying out this test.

Sample Preparation

Send serum samples (2 mL) to the SAS laboratory. Request the assay of testosterone and 5 α -DHT.

Reference Ranges

Serum concentrations of testosterone and 5 α -DHT are very variable within the first 6 months of life and through puberty. Baseline values may be at the limit of sensitivity and the testosterone/5 α -DHT ratio may be difficult to establish with confidence. In pre-pubertal patients values should be assessed before and after treatment with hCG.

Testosterone/5 α -Dihydrotestosterone ratio following a stimulation test:

	Unaffected	Affected
Less than 6 months of age:	<20	>20
Less than 6 months of age (after hCG):	<10	>20
6 months - puberty:	<20	<20
6 months - puberty (after hCG):	<27	>27
Adult males:	<17	>25
Adult males (after hCG):	<17	>20

The SAS laboratory will undertake assay of both testosterone and dihydrotestosterone in order to be able to advise on the interpretation of the testosterone/5 α -DHT ratio.

Centres offering this assay

Leeds, London (St. Thomas').

References

Pang S, Levine LS, Chow D et al. Dihydrotestosterone and its relationship to testosterone in infancy and childhood. *J Clin Endocrinol Metab* 1979; **48**: 821-826.

Wilson JD, Griffin JE, George JW, Lestin M. The role of gonadal steroids in sexual differentiation. *Rec Progr Hormone Res* 1981; **37**: 1-39.

Erythropoietin (serum)

Introduction

Erythropoietin (EPO) is a glycoprotein hormone that is the main stimulus for red cell production in the body. Human EPO has a molecular weight of 30.4 kilodaltons and is composed of 165 amino acids. It is heavily glycosylated with a carbohydrate moiety of approximately 40% and is secreted predominantly by the kidneys in response to hypoxia.

Clinical Indications

1. Differential diagnosis of anaemia

Most anaemic patients have appropriately elevated serum EPO that is dependent on the degree and type of anaemia. Aplastic anaemia, haemolytic anaemia and anaemia due to iron deficiency result in increased serum EPO concentrations. In patients with anaemia associated with chronic renal failure the serum EPO concentration is usually inappropriately low due to reduced EPO production by the kidneys.

2. Differentiation between primary and secondary polycythaemia

Patients with polycythaemia rubra vera (primary polycythaemia) typically present with normal or below normal serum EPO whereas those with secondary polycythaemia brought on by hypoxia induced congestive heart failure or chronic obstructive pulmonary disease present with elevated serum EPO concentrations.

3. Monitoring EPO therapy

Treatment with recombinant EPO has been shown to be successful in diseases such as anaemia of chronic renal failure, multiple myeloma and myelodysplastic syndromes and anaemia of prematurity. Monitoring serum EPO concentrations pre and during therapy reduces toxicity and ensures adequate therapeutic concentrations are achieved.

Method information

The method used is a solid phase chemiluminescent immunometric assay for use on the DPC Immulite 2000 analyser.

Patient Preparation

None.

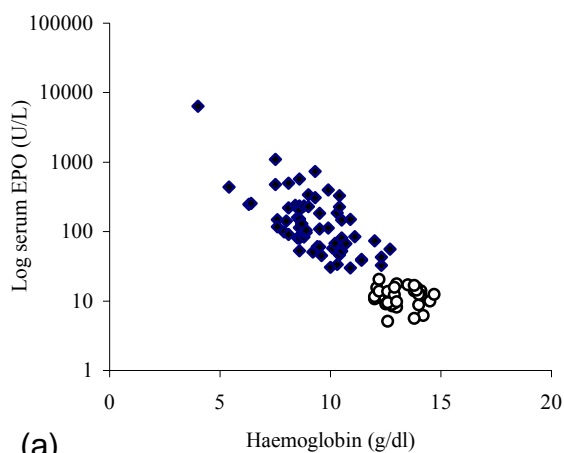
Sample Preparation

Collect blood into a tube containing no additives and allow to clot. Separate the serum promptly and store at 4°C. The sample is stable for 7 days at 4°C. For longer storage it is recommended that samples are stored at -20°C, repeat freeze thawing should be avoided. A minimum volume of 500 µl serum is required for analysis.

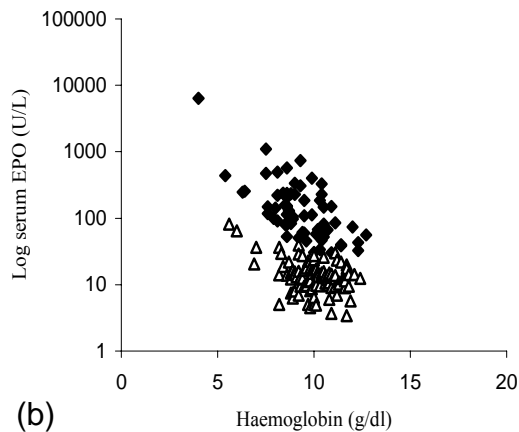
External samples can be transported unfrozen by 1st class post or courier to the laboratory. State all relevant clinical information including whether the patient is receiving recombinant EPO therapy and current haemoglobin level. A customised laboratory form is available.

Reference range

The normal range for a serum EPO concentration is 5 – 25 U/L for an individual with a haemoglobin level within normal limits. The following graphs illustrate whether the serum EPO concentration is appropriate for the haemoglobin level.



(a)



(b)

Serum EPO concentrations in patients with non-renal anaemia ◆ compared to (a) normal controls o and (b) renal anaemia Δ

Quality Assessment

There is no external quality assessment scheme but there is participation in a sample exchange scheme.

Centre offering this test

London (King's).

References

Marsden JT, Day P, Ellis R, Marwah s, Savage G, Sinclair C. A sample distribution program for erythropoietin. Clin Lab Haem 2006; 28: 228-232.

Marsden JT. Erythropoietin – measurement and clinical applications. Ann Clin Biochem 2006; 43: 97-104.

α -Fetoprotein (serum)

Clinical Indications

Diagnosis and management of primary liver cancer, gonadal tumours, rare carcinomas (particularly of the gastrointestinal tract) and in certain non-neoplastic hepatic diseases.

If a gonadal tumour is suspected, measurement of β -hCG should also be requested.

Patient Preparation

None. Take blood (5 mL) into a plain tube.

To facilitate assessment of therapy, it is important that a sample be obtained before treatment is initiated.

Sample Preparation

Send serum (0.5 mL) to the SAS laboratory. Record on the SAS request form clinical diagnosis, whether the patient is hepatitis antigen or antibody positive, information on radio- or chemotherapy and, if appropriate, the result of CT or ultrasound liver scan.

Reference Range

Adults: <10 kU/L

In primary liver cancer, 80% of patients with hepatocellular cancer have serum α -fetoprotein >300 kU/L. Some chemotherapeutic agents, e.g. cis-platinum, cause elevation of α -fetoprotein values, which usually reach a consistent plateau, then may take many months to decline to normal after completion of therapy.

Quality Assessment

UK NEQAS.

Centre offering this assay

London (Charing Cross).

References

Coppack S, Newlands ES, Dent J, Mitchell H, Goka G, Bagshawe KD. Problems of interpretation of serum concentrations of alpha-foetoprotein (AFP) in patients receiving cytotoxic chemotherapy for malignant germ cell tumours. *Br J Cancer* 1983; **48**, 335-340.

Gastrin

(Plasma/Serum)

Clinical Indication

Diagnosis of Zollinger Ellison Syndrome (gastrin producing tumours).

Patient Preparation

With the patient at rest after an overnight fast, take blood (10 mL) into a plain tube or collect with a syringe and needle.

H₂ blockers should be stopped for 72 hours and omeprazole for two weeks before blood is taken.

Sample Preparation

For Guildford (other gut hormones are not measured in Guildford):

Collect or transfer blood (10 mL) into a plain tube (tubes containing gel can be used). The sample should be rushed to the lab, separated in a refrigerated centrifuge and stored at -20°C. Send 1 mL of serum and ensure that it remains frozen during transport.

For Hammersmith:

Serum prepared as above is acceptable for gastrin alone **however if the request is part of a gut hormone screen or if any other part of the screen may be required in retrospect then the blood MUST be prepared as follows.**

Immediately add Trasylol (aprotinin, 0.2mL, 2000 kIU/mL blood). Mix by inversion; place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Send at least 1 mL plasma to the SAS laboratory for glucagon analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

NB Visible haemolysis invalidates results for gastrin.

To aid with interpretation, record on the SAS request form the patient's serum calcium and urea values, a list of all drugs currently administered, details of any gastric surgery, and basal and stimulated acid output if available.

Reference Ranges

Reference ranges are supplied with results, please contact the SAS laboratory if you need further information.

Quality Assessment

UK NEQAS.

Centres offering this assay

Guildford, London (Hammersmith).

Glucagon (pancreatic) (plasma)

Clinical Indication

Preoperative diagnosis of a glucagon-producing tumour of the pancreas in patients with diabetes and a characteristic skin rash (necrolytic migratory erythema).

Patient Preparation

With the patient at rest after an overnight fast take blood (10mL) into a Lithium Heparin vacutainer or, using a syringe and needle, add the blood to a Lithium Heparin tube.

Sample Preparation

Immediately add Trasylol (aprotonin, 0.2mL, 2000 kIU/mL blood) to the lithium heparin tube. Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result. Send at least 1 mL plasma to the SAS laboratory for glucagon analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

Reference Ranges

Adults (fasting)	<50 pmol/L
Adults with a glucagonoma	>200 pmol/L

Quality Assessment

There are no external quality assessment schemes for this analyte.

Centre offering this assay

London (Hammersmith).

References

Bloom SR, Long RG. Radioimmunoassay of Gut Regulatory Peptides London WJ Saunders 1982.

Bloom SR, Hammond P. Endocrinology of the Gastrointestinal Tract. In Clinical Endocrinology ed Besser GM and Thorner MO. Wolfe London 1994.

Human Chorionic Gonadotrophin - β -hCG (CSF, serum, urine)

Clinical Indications

1. Diagnosis of choriocarcinoma and germ cell tumours of the ovary, testis or mediastinum and monitoring response to therapy.
2. Monitoring other rare cancers which produce this gonadotrophin ectopically.
3. Detection of metastases to the brain from choriocarcinoma or germ cell tumours.

Applications

When hCG is used to diagnose early pregnancy, the concentration will double in approximately 48h. In both trophoblastic tumours and ectopic pregnancies, there is a slower rise with hCG values lower than for a comparable stage of normal pregnancy. A fall in concentration will suggest a failing pregnancy or miscarriage.

Patients confirmed as having a hydatidiform mole should be registered with the appropriate follow-up Centre (Charing Cross Hospital, London or Royal Hallamshire Hospital, Sheffield).

1, 2. In trophoblastic disease and germ cell tumours.

In these conditions hCG may be secreted as the intact dimer, free β -subunit or fragments such as β -core fragment (which is cleared very rapidly from serum and detectable only in urine). In addition, the β -subunit may be "nicked" i.e. lose specific sections of the peptide chain and the C-terminal may be missing altogether. These changes will affect most commercial assays which were developed principally for measuring hCG in pregnancy and which require two unaltered epitopes to complete the "sandwich".

The antibody used in this radioimmunoassay is directed to a single epitope on the β -subunit, detects both free β , intact hCG and β -core fragment, is not affected by "nicks" and cross-reacts less than 0.25% with luteinising hormone of pituitary origin.

Secretion of hCG declines during successful therapy; renewed secretion provides evidence of recurrence.

3. Cerebral metastases.

Intracerebral tumours secreting hCG can be detected from comparison of β -hCG concentrations in peripheral serum and cerebrospinal fluid.

Patient Preparation

None.

1,2. Diagnosis of germ cell tumours or ectopic production of hCG.

Take blood (5 mL). For management of these conditions, take blood (5 mL) before surgery or therapy and within 3 days of initiation of treatment and at regular intervals during follow-up.

3. Cerebral metastases.

Take peripheral blood (5 mL) and cerebro-spinal fluid (2 mL).

Patients registered for the hydatidiform mole follow-up service will be instructed to collect blood and/or urine at the required intervals. Containers and instructions will be provided.

Sample Preparation

Serum: Collect serum and store at -20°C .

Urine: Collect urine from an early morning voiding in the tube provided, which contains merthiolate (final concentration 0.01% w/v). Store at -20°C .

Cerebrospinal fluid: Collect without anticoagulant. Store at -20°C .

Send serum (not less than 1 mL), cerebro-spinal fluid (2 mL) or urine (2 mL) to the SAS laboratory. Record on the SAS request form the clinical details and an estimate of the hCG level if known (to facilitate dilution and avoid unnecessary delay).

Reference Ranges

Adult males or non-pregnant females:

Serum: <5 IU/L;

Urine: <25 IU/L;

CSF: <5 IU/L.

In the absence of brain metastases, the ratio of hCG concentrations in serum and CSF is $>60:1$ (mean 280:1). Ratios of $<60:1$ are highly suggestive of the presence of intracerebral tumour tissue secreting hCG, unless serum hCG is falling rapidly in response to therapy.

Quality Assessment

UK NEQAS.

Centre offering this assay

London (Charing Cross).

17 α -Hydroxyprogesterone (saliva)

Clinical Indication

Monitoring corticosteroid replacement therapy in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

The use of saliva permits collection of multiple samples in a non-invasive manner in a stress-free (home) environment. Corticosteroid dose can be adjusted until salivary concentrations throughout the day fall within defined limits.

Patient Preparation

None, but saliva must be collected in the following manner:

1. Rinse mouth thoroughly with water to remove food debris;
2. After 5 - 10 min, allow saliva to collect in floor of mouth;
3. Gently express saliva into the collecting vial
4. Repeat steps 2 & 3 until at least 1 mL saliva (excluding froth) has been collected.

Saliva may be obtained from a very young child by gentle suction.

Collect saliva at the following times:

First sample	Before taking early morning dose of steroid
Second sample	Between 11.00h and 13.00h
Third sample	Between 15.00h and 17.00h
Fourth sample	Before taking late night dose of steroid

Sample Preparation

During collection of saliva at home, store the samples at 4°C. Store samples in the laboratory at -20°C before despatch. Send saliva samples (minimum of 1 mL) to the SAS laboratory.

Reference Ranges

The following concentrations are associated with treatment judged to be adequate by accepted criteria:

<u>Time</u>	<u>Concentrations of 17-hydroxyprogesterone</u>
0800h	650 – 2800 pmol/L
1200h	350 – 1700 pmol/L
1600h	220 – 1500 pmol/L
2200h	170 – 1100 pmol/L

Quality Assessment

There is no external quality assessment scheme for this analyte.

Centres offering the assay

Cardiff, Leeds.

References

Miller WL. Genetics, diagnosis and management of 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1994; 78: 241 -6.

Riad-Fahmy D, Read GF, Walker R, Griffiths K. Steroids in saliva for assessing endocrine function. *Endocr Revs* 1981; 3: 367-95.

17 α -Hydroxyprogesterone (serum)

Clinical Indications

1. Diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency.
2. Monitoring the response to corticosteroid therapy in congenital adrenal hyperplasia due to 21-hydroxylase deficiency.
3. Identification of heterozygotes for congenital adrenal hyperplasia and diagnosis of mild congenital adrenal hyperplasia (also referred to as late-onset and cryptic forms).

17 α -Hydroxyprogesterone is an intermediate in the biosynthesis of cortisol. Deficiency of either 11 β - or 21-hydroxylase activities leads to an increased concentration of 17 α -hydroxyprogesterone in the peripheral circulation. This test is less valuable in 11 β -hydroxylase deficiency, where 11-deoxycortisol is the analyte of choice. 'Functional' deficiencies of 21-hydroxylase and 11 β -hydroxylase have been described.

Patient Preparation

1. *Diagnosis of congenital adrenal hyperplasia.*

Take blood (1 mL) into a plain tube before any emergency administration of corticosteroids and preferably in the early morning.

The SAS offers 2 assays: extraction RIA and GC-MS)

Radioimmunoassay - Samples for RIA tests should not be taken from newborn infants until more than 48h after birth because there is some interference in the assay from fetal, maternal and placental steroids.

GC-MS analysis -If the samples are being sent for GC-MS analysis then samples from the end of the first day are acceptable for assay since no interfering steroids are detected.

N.B All new cases of congenital adrenal hyperplasia should be confirmed by urine steroid profiling.

2. *Monitoring response to treatment.*

Take blood (1 mL) between 08.00h and 09.00h before initiation of treatment for the day and again 2h after the first dose of corticosteroid. Further samples may be taken through the day. Record the time of any therapy. Adequacy of treatment may be better judged if combined with the measurement of androstenedione.

3. *Identification of heterozygotes and diagnosis of mild congenital adrenal hyperplasia.*

In a female patient with menstrual cycles, the test should be carried out between 09.00h and 10.00h in the first five days following menstruation. Take blood (1 mL) for baseline value. Inject adrenocorticotrophin (ACTH 1-24, Tetracosactrin Ciba, 0.25 mg i.m.). Take blood (1 mL) 1 h after injection.

Sample Preparation

Send serum samples (0.5 mL) to the SAS laboratory. Record on the SAS request form gestational age at delivery, plasma electrolytes and any genital abnormality. For urgent requests (e.g. acutely ill infants) contact the laboratory before despatching the sample.

Reference Ranges

(Age related reference ranges for children may be available from each Centre.)

	RIA	GC-MS
Infants (unstressed)	<13	< 8 nmol/L
Infants (stressed)	<40	
Adult males	2-9 nmol/L	3 – 10 nmol/L
Adult females – follicular phase	2 – 6 nmol/L	1 – 4.5 nmol/L
Adult females – luteal phase	> 6 nmol/L	< 7 nmol/L
Patients with untreated 21-hydroxylase deficiency	Usually > 100 nmol/L	> 100 nmol/L

Values 1 hour after ACTH:

Unaffected adults 3-30 nmol/L (RIA); < 11 nmol/L (GC-MS)

Heterozygotes for 21-hydroxylase deficiency 6 – 44 nmol/L (RIA); 5 – 50 nmol/L (GC-MS)

Heterozygotes for mild 21-hydroxylase deficiency 63 – 470 nmol/L (RIA); 60 – 800 nmol/L (GC-MS)

Quality Assessment

UK NEQAS (RIA and GC-MS); BIORAD (GC-MS only).

Centres offering the assay

Cardiff*, Leeds*, London (St Thomas')*, London (UCLH)**

* RIA assay

** GC-MS

References

Kuttens F, Couillin O, Girard F et al. Late-onset adrenal hyperplasia in hirsutism. *NEJM* 1985; **313**: 224-31.

New MI, Lorenzen F, Lerner AJ et al. Genotyping steroid 21-hydroxylase deficiency: Hormonal reference data. *J Clin Endocrinol Metab* 1983; **57**: 320-6.

Wallace AM. Analytical support for the detection and treatment of congenital adrenal hyperplasia. *Ann Clin Biochem* 1995; **32**: 9-27.

Avivi I et al. Overdiagnosis of 21-hydroxylase late onset congenital adrenal hyperplasia: Correlation of corticotropin test and human leucocyte antigen typing. *Fert Steril* 1996; **66**: 557-63.

Ismail AA, Cawood M, Ufodiama EB, Jones ST. Limitations of 17-hydroxyprogesterone in investigating neonatal hyponatraemia. *Ann Clin Biochem*. 2004; **41**: 245-7.

Wudy SA et al. 17 alpha-hydroxyprogesterone, 4-androstenedione, and testosterone profiled by routine stable isotope dilution/gas chromatography-mass spectrometry in plasma of children. *Pediatr Res*. 1995; **38**: 76-80.

25-Hydroxyvitamin D (serum)

Clinical Indications

1. Investigation of disorders of mineral metabolism in children and adults
2. Diagnosis of vitamin D deficiency and insufficiency in children and adults.
3. Investigation of suspected vitamin D intoxication.

25-Hydroxyvitamin D (25-hydroxycholecalciferol), the immediate precursor of the biologically active renal metabolite 1,25-dihydroxyvitamin D is the major circulating metabolite of vitamin D. Measurement provides an index of vitamin D status for the investigation of patients with suspected osteomalacia, rickets or obscure hyper- or hypocalcaemia.

Patient Preparation

None. No special dietary restriction is necessary. Take blood (5mL) into a red-topped vacutainer.

Sample Preparation

Frozen samples are not required by Manchester or Newcastle.

Send serum (2 mL) by first-class post to the SAS laboratory.

If sample has to be frozen, freeze the serum (-20⁰C) promptly and ensure the sample remains frozen during transport.

Reference Range

There is some seasonal variation. Normal ranges are provided with results.

Quality Assessment

DEQAS.

Centres offering this assay

Cardiff, London (Hammersmith), Manchester, Newcastle.

References

Iqbal SJ. Vitamin D metabolism and the clinical aspects of measuring metabolites. *Annals Clin Biochem* 1994; **31**: 109-124.

Lips P, Chapuy MC, Dawson-Hughes B, Pols HAP, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements *Osteoporosis Int* 1999; **9**: 394-397.

Heaney RP. Vitamin D: how much do we need, and how much is too much? *Osteoporosis Int* 2000; **11**: 553-555.

Turpeinen U, Hohenthal U, Stenman, U-H. Determination of 25-hydroxyvitamin D in serum by HPLC and immunoassay *Clin Chem* 2003; **49(9)**: 1521-1524.

Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, DeLuca HF, Drezner MK. Assay variation confounds the diagnosis of hypovitaminosis D: A call for standardization. *J Clin Endocrinol Metab* 2004; **89(7)**: 3152-3157.

Carter GD, Carter R, Jones J, Berry JL. How accurate are assays for 25-hydroxyvitamin D? Data from the International Vitamin D External Quality Assessment Scheme. *Clin Chem* 2004; **50**: 2195-2197.

Grant WB and Holick MF. Benefits and requirements of vitamin D for optimal health: A review. *Alternative Medicine Review* 2005; **10**: 94-111.

Heaney RP. The vitamin D requirement in health and disease. *J Steroid Biochem & Molec Biol* 2005;97: 13-19.

Dawson -Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporosis Int* 2005; **16**: 713-716.

Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: Implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005; **135**: 317-322.

Glendenning P, Taranto M, Noble JM, Musk AA, Hammond C, Goldswain PR, Fraser WD, Vasikaran SD. Current assays overestimate 25-hydroxyvitamin D3 and underestimate 25-hydroxyvitamin D2 compared with HPLC: need for assay-specific decision limits and metabolite-specific assays. *Ann Clin Biochem* 2006; **43**: 23-30.

25-Hydroxyvitamin D with 1,25-Dihydroxyvitamin D (serum)

Clinical Indication

Investigation of disorders of mineral metabolism.

1,25-Dihydroxyvitamin D (1,25-dihydroxycholecalciferol, calcitriol), the active hormonal metabolite of vitamin D, is formed in the kidney. This renal metabolite is tightly regulated and synthesis is enhanced by the presence of parathyroid hormone, hypocalcaemia and hypophosphataemia and is suppressed by hypercalcaemia, hyperphosphataemia and impaired renal function. Thus measurement may be useful in renal disease, in primary and secondary hyperparathyroidism and hypoparathyroidism and in distinguishing the various types of vitamin D resistant states from vitamin D deficiency. In certain pathological conditions 1,25-dihydroxyvitamin D may be synthesised at non-renal sites e.g. activated macrophages and assays may also assist in investigation of malignancy-associated hypercalcaemia and sarcoidosis.

25-Hydroxyvitamin D is always measured on the same sample, since results for 1,25-dihydroxyvitamin D cannot be interpreted without this information. The assays measure both the D3 and D2 forms of the vitamin.

Patient Preparation

None. No special dietary restriction is necessary.

Sample Preparation

Send serum (2 mL) by first-class post to the SAS laboratory. If sample has to be frozen (see 25-hydroxyvitamin D section), freeze the serum (-20°C) promptly and ensure the sample remains frozen during transport.

Reference Ranges

25-hydroxyvitamin D shows some seasonal variation. Normal ranges are provided with results.

1,25-Dihydroxyvitamin D: 20-55 pg/mL (48-120 pmol/L)

Centre offering this assay

Manchester.

References

Mawer EB, Berry JL, Cundall JP, Still PE and White A. A sensitive radioimmunoassay that is equipotent for ercalcitriol and calcitriol (1,25_dihydroxyvitamin D2 and D3). *Clin Chim Acta* 1990; **190**: 199-210.

Mawer EB and Berry JL. The biochemistry of calcium regulation. In *The Measurement of Metabolic Bone Disease*. FI Tovey and T Stamp (eds), Parthenon Publishing, Carnforth, Lancs, 1995; Chapter 5, pp 49-75.

Berry JL, Davies M and Mee AP (2002) Vitamin D metabolism, rickets and osteomalacia. In *Seminars in Musculoskeletal Radiology*, D Karasick, ME Schweitzer and JE Adams (eds), Thieme Medical Publishers Inc NY 2002; 6 (3) pp ?

Insulin

(plasma, serum)

Clinical indication

1. Differential diagnosis of documented spontaneous hypoglycaemia.

The measurement of both insulin and C-peptide is advisable in the investigation of hypoglycaemia since in some cases of insulinoma, insulin levels may be low or marginally inappropriate whereas C-peptide is almost always unequivocally raised¹⁻³. Both measurements are essential for the identification of factitious insulin administration. Insulin assays vary in their cross-reactivity to recombinant insulin analogues however those used by the SAS laboratories can detect such preparations^{4,5}. Further tests, such as those for ketones, proinsulin, sulphonylureas, growth hormone and the insulin-like growth factors may also be required to complete the diagnostic process.

Patient preparation

Hypoglycaemia, spontaneous or whilst fasting, must first be established by regular monitoring of blood glucose levels. Blood (10 mL) collected in a plain vacutainer or syringe, when the blood glucose concentration (confirmed by laboratory analysis) is less than 2.2 mmol/L (or less than 2.5 mmol/L in patients over 60 years), should be used for the tests. Vacutainers (7 mL, red top) may be used for blood collection.

Sample preparation

Transfer the blood to a plain tube. Separate the serum preferably within 30 min of collection and freeze at -20°C . Larger volumes of sample are required for additional studies such as IGF-1 measurements or sulphonylurea identification.

Small blood samples from neonates should be transferred to a heparin tube so that the maximum volume of plasma may be collected. A minimum volume of 0.2 mL should be stored at -20°C . Visible haemolysis may invalidate the result.

Send samples to the SAS laboratory and ensure the samples remain frozen during transport. Record the blood glucose concentration on the request form.

Reference range

An interpretation of the result(s) will be provided where sufficient information accompanies the request. For further clarification please contact the SAS laboratory.

Quality Assessment

UK NEQAS.

Centres offering this assay

Cardiff, Guildford, London (Hammersmith).

References

1. Clark PM. Assays for insulin, proinsulin(s) and C-peptide. *Ann Clin Biochem* 1999; **36**: 541-564.
2. Marks V, Teale JD. Hypoglycaemia in the adult. In: Hypoglycaemia. Gregory JW, Aynsley-Green A (eds). Baillière's Clinical Endocrinology and Metabolism 1999; **7**: 705-729.
3. Marks V, Teale JD. Investigation of hypoglycaemia. *Clin Endocrinol* 1996; **44** : 133-136.
4. Owen WE, Roberts WL. Cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. *Clin Chem* 2004; **50**: 257-259.
5. Heald AH, Bhattacharya B et al. Most commercial assays fail to detect recombinant insulin analogues. *Ann Clin Biochem* 2006; **43**: 306-308.

Insulin Antibodies (serum)

Clinical Indications

1. Investigation of hypoglycaemia associated with antibodies to either endogenous or more commonly, exogenous insulin (particularly animal preparations).
The presence of antibodies can cause impaired glucose tolerance and attacks of reactive hypoglycaemia.
2. Detection of insulin autoimmunity can be useful in the assessment of the early stages of diabetes mellitus.

Patient Preparation

Take blood (10 mL) into a plain vacutainer or syringe either during hypoglycaemia (where this occurs) or after an overnight fast.

Sample Preparation

Transfer the blood to a plain tube and separate the serum promptly. Store at -20°C or send to the SAS laboratory by first-class post.

Method Information

ELISA.

Reference Range

A positive result is abnormal although a semi-quantitative estimate of antibody titre will be provided.

Centre offering this assay

Guildford.

Reference

Redmon JB, Nuttall FQ. Autoimmune hypoglycaemia. *Endocrinology and Metabolism Clinics of North America* 1999; **28**: 603-618.

Insulin-like Growth Factor I (IGF-I) (serum)

Introduction

Insulin-like growth factor 1 (IGF-1) is part of a group of peptides including IGF-2, the IGF binding proteins (IGFBP1 – 6) and acid labile subunit, whose principle role is to co-ordinate growth and metabolism. IGF-1 is primarily synthesized in the liver and under the control of growth hormone (GH), though paracrine and autocrine functions are likely. IGF-1 has a long half-life and serum concentrations show fewer fluctuations than serum GH.

Clinical Indications

1. Diagnosis and monitoring treatment of acromegaly.

When measured in a random sample, serum insulin-like growth factor I (IGF-I) concentration correlates more closely with disease status than does serum growth hormone (GH) concentration.

2. Investigation of short stature in children.

Measurement of IGF-I in conjunction with the assay of growth hormone during a stimulation test, may assist in establishing the cause of retarded growth.

3. Differential diagnosis of spontaneous hypoglycaemia.

When suppressed concentrations of insulin, C-peptide, ketones and GH are associated with severe hypoglycaemia, the measurement of IGF-I and IGF-II is indicated. An elevated IGF-II to IGF-I molar ratio is consistent with the diagnosis of a non-islet cell tumour.

Method information

Acid ethanol extraction, in-house RIA (Birmingham)

Displacement immunometric assay (Guildford, London (St. Thomas'), Newcastle)

Patient Preparation

1,2 Investigation of acromegaly and short stature.

Collect blood (10 mL) into a plain vacutainer or syringe.

3. Differential diagnosis of spontaneous hypoglycaemia.

Collect blood (10 mL) during a hypoglycaemic episode into a plain vacutainer (see sections on insulin and C-peptide).

Sample Handling

Transfer the blood to a plain tube and allow to clot. Use plain tubes for sample collection. Clot accelerators and gel tubes should not be used. Separate the serum promptly and store at -20°C.

1, 2 Investigation of acromegaly and short stature.

Send 1 mL serum to the SAS laboratory. Record on the request form the following details: age and sex; clinical information on evidence of hypothyroidism; diabetes; nutritional status; liver function; details of current treatment.

3 Differential diagnosis of spontaneous hypoglycaemia.

Send 3 mL serum to the SAS laboratory, and ensure the sample remains frozen during transport if insulin and C-peptide measurement is also required (see Insulin and C-peptide sections).

Result Interpretation

Reference ranges are age and gender dependent. Where these details are provided, the relevant reference range will be quoted.

Serum concentrations of IGF-1 may be low due to poor nutrition and hepatic failure.

Quality Assessment

UK NEQAS.

Centres offering this assay

Birmingham, Guildford, London (St. Thomas'), Newcastle.

References

Peacey S R, Shalet S M. Insulin-like growth factor 1 measurement in diagnosis and management of acromegaly. *Ann Clin Biochem* 2001; **38**: 297-303.

Rosen CJ. Serum insulin-like growth factors and insulin-like growth factor-binding proteins: clinical implications. *Clin Chem* 1999; **45**: 1384-1390

Shakespear RA, Lynch SS, Rudd BT, Williams JW, White DA. Serum IGF-1 assays: which sample preparation method? Proceedings of the ACB National Meeting 1993; C11.

Insulin-like Growth Factor II (IGF-II) (serum)

Introduction

In some cases of non-islet cell tumour where severe hypoglycaemia is present, IGF-II produced by the tumour has been implicated as the hypoglycaemic agent. The diagnosis is based on an elevated IGF-II to IGF-I molar ratio in association with suppressed insulin, C-peptide, ketones and GH.

Clinical Indication

Differential diagnosis of spontaneous hypoglycaemia (see also section on IGF-I).

Method Information

In-house radioimmunoassay.

Patient Preparation

Hypoglycaemia, spontaneous or whilst fasting, must first be established by regular monitoring of blood glucose levels. Blood (10 mL) collected in a plain vacutainer or syringe, when the whole blood glucose concentration (confirmed by laboratory analysis) is less than 2.2 mmol/L (or less than 2.5 mmol/L in patients over 60 years), should be used for the tests. A minimum volume of 0.5 mL serum should be stored at -20°C.

Sample Handling

Transfer the blood to a plain tube. Separate the serum preferably within 30 min of collection and freeze at -20°C. Larger volumes of sample (3 mL) are required for additional studies such as insulin, C-peptide and IGF-1 measurements or sulphonylurea identification. Visible haemolysis may invalidate the result.

Send samples to the SAS laboratory and ensure they remain frozen during transport if insulin and C-peptide analysis is also required. Record the blood glucose concentration on the request form. Follow-up samples (collected during or after treatment) requiring only IGF-II measurements may be sent by first class post.

Result Interpretation

The SAS Laboratory will provide appropriate reference data and an interpretation of results based on relevant biochemical and clinical information.

Quality Assessment

No external quality assessment scheme is available. Sample exchange occurs with other specialist laboratories.

Centre offering this assay

Guildford.

Reference

Marks V, Teale JD. Tumours producing hypoglycaemia. *Diabetes/ Metabolism Revs.* 1991; 7: 79-91.

Neurotensin (plasma)

Clinical Indication

Diagnosis of pancreatic endocrine tumours.

In about 10% of patients with a tumour which secretes vasoactive intestinal peptide (VIP), the plasma concentration of neurotensin is found to be elevated. A very small proportion of patients have a pure neurotensinoma. In these cases the patient is asymptomatic.

Patient Preparation

With the patient at rest after an overnight fast take blood (10mL) into a Lithium Heparin vacutainer or, using a syringe and needle add the blood to a Lithium Heparin tube.

Sample Preparation

Immediately add Trasylol (aprotinin, 0.2mL, 2000 kIU/mL blood). Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result.

Send at least 1 mL plasma to the SAS laboratory for neurotensin analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

Reference Ranges

Adults (fasting) <100 pmol/L

Adults with endocrine tumours producing somatostatin >500 pmol/L

Quality Assessment

There are no external quality assessment schemes or other centres offering this assay for sample swapping schemes to occur.

Centre offering this assay

London (Hammersmith).

References

Bloom SR, Long RG. Radioimmunoassay of Gut Regulatory Peptides. London WJ Saunders 1982

Bloom SR, Hammond P. Endocrinology of the Gastrointestinal Tract. In Clinical Endocrinology ed Besser GM and Thorner MO. Wolfe London 1994

Pancreatic Polypeptide

(plasma)

Clinical Indication

Diagnosis of pancreatic endocrine tumours.

Pancreatic polypeptide (PP) production is most commonly associated with tumours producing vasoactive intestinal polypeptide and with carcinoid syndrome and, less commonly, with insulinomas and gastrinomas.

Patient Preparation

With the patient at rest after an overnight fast take blood (10mL) into a Lithium Heparin vacutainer or, using a syringe and needle add the blood to a Lithium Heparin tube.

Sample Preparation

Immediately add Trasylol (aprotonin, 0.2mL, 2000 kIU/mL blood). Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result. Send at least 1 mL plasma to the SAS laboratory for pancreatic polypeptide analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

Reference Ranges

Adults (fasting) <300 pmol/L

Adults with endocrine tumours producing PP >300 pmol/L

About 50 % of patients with pancreatic endocrine tumours have elevated values for pancreatic polypeptide. Adults with strong vagal tone or after a meal may have values >300 pmol/L. Pancreatic polypeptide is also found to be at the upper limit of normal in elderly patients.

Quality Assessment

There is no external quality assessment scheme.

Centre offering this assay

London (Hammersmith).

References

Bloom SR, Long RG. Radioimmunoassay of Gut Regulatory Peptides London WJ Saunders 1982

Bloom SR, Hammond P. Endocrinology of the Gastrointestinal Tract. In Clinical Endocrinology ed Besser GM and Thorner MO. Wolfe London 1994

Parathyroid Hormone (Intact) (plasma)

Clinical Indications

1. Differential diagnosis of hypercalcaemia
2. Assessment of parathyroid activity in patients with chronic renal disease.
3. Monitoring of patients with hyperparathyroidism secondary to Vitamin D deficiency or malabsorption.

The high sensitivity assays now available permit good discrimination between normal subjects, patients with primary hyperparathyroidism and patients with hypercalcaemia of malignancy. Measurement of PTH also enables identification of renal patients at risk of osteodystrophy and provides the means of monitoring their response to therapy.

Patient Preparation

There is a nocturnal rise in PTH and samples are best collected in the morning after 7am and preferably after an overnight fast. Collect blood (4-5 mL) by venepuncture into an EDTA vacutainer with or without gel separator.

Sample Preparation

Transfer the sample to the laboratory at the earliest opportunity. Separate the plasma, preferably in a refrigerated centrifuge, and store the plasma at -20°C in a plastic tube. The plasma should be frozen within 4 hours of venepuncture. Marked haemolysis invalidates the result. Send at least 1 mL plasma to the SAS laboratory. Record on the SAS form the serum calcium concentration. Ensure the sample remains frozen during transport.

Reference Range

Each SAS laboratory will supply reference ranges with the result. Interpretation may be dependant on provision of a corresponding calcium result.

Quality Assessment

UK NEQAS.

Centres offering this assay

Cardiff, London (Hammersmith), Newcastle.

References

Gupta MK, Koo D. Endocrine disorders (parathyroid hormone). *Anal Chem* 1993, 15, **65** (12): 378R-381R.

Slatopolsky E, Delmez JA. Pathogenesis of secondary hyperparathyroidism. *Nephrol Dial Transplant* 1996; **11** Suppl 3: 130-135.

Ashby JP, Newman DJ, Gow SM. Clinical application of intact parathyroid hormone assays. *Ann Clin Biochem* 1997; **34**: 588-598.

Blumsohn A, Hadari AA. Parathyroid hormone: what are we measuring and does it matter. *Ann Clin Biochem* 2002; **39**: 169-172

Proinsulin (serum)

Clinical Indication

Differential diagnosis of documented spontaneous hypoglycaemia.

Autonomous secretions of some, but not all, insulinomas contain an abnormally high proportion of immunoreactive proinsulin. An elevated blood level can confirm this diagnosis but a low (normal) result cannot exclude it.

Patient Preparation

Hypoglycaemia, spontaneous or whilst fasting, must first be established by regular monitoring of blood glucose levels. Blood (10 mL) collected in a plain vacutainer or syringe, when the whole blood glucose concentration (confirmed by laboratory analysis) is less than 2.2 mmol/L (or less than 2.5 mmol/L in patients over 60 years), should be used for the tests. Vacutainers (7 mL, red top) may be used for blood collection.

Sample Preparation

Transfer the blood to a plain tube. Separate the serum, preferably within 30 min of collection and freeze at -20°C . Larger volumes of sample (2 mL) are required for additional studies such as IGF-1 measurements or sulphonylurea identification. A minimum volume of 0.2 mL should be stored at -20°C . Visible haemolysis may invalidate the result.

Send samples to the SAS laboratory and ensure they remain frozen during transport. Record the blood glucose concentration on the request form.

Reference Range

An interpretation of the result(s) will be provided where sufficient information accompanies the request. For further clarification please contact the SAS laboratory.

Quality Assessment

No external quality assessment scheme is yet available. Sample exchange occurs between laboratories.

Centre offering this assay

Guildford.

Reference

Marks V, Teale JD. Investigation of hypoglycaemia. *Clin Endocrinol* 1996; **44**: 133-136.

Renin Activity (plasma)

Clinical Indications

1. Diagnosis of, and differentiation between, primary and secondary causes of hyper- or hypoaldosteronism.
2. Management of renal artery stenosis.
3. Diagnosis and location of renin secreting tumours.
4. Monitoring mineralocorticoid replacement therapy.

Applications

1. Hyper- or hypoaldosteronism.

The investigation of disorders of aldosterone production requires both aldosterone and plasma renin activity (PRA) measurements.

For detailed protocols, please refer to the Aldosterone section, Patient Preparation.

2. Renal artery stenosis.

The measurement of PRA in renal vein samples from both kidneys can assist in the diagnosis of renal artery stenosis. The extent to which renal vein PRA is asymmetrical provides a guide to the likelihood that the hypertension associated with this condition will be corrected successfully by surgery. Measurement of PRA in the peripheral circulation is of no value since levels may be normal or raised.

3. Renin secreting tumour.

Hypertensive patients with very high levels of PRA in the peripheral circulation, and in whom chronic renal disease and renal artery stenosis have been excluded, may have a renin secreting tumour. These tumours, often too small to be visualised by renal arteriography, can be located by the increased PRA in samples from the renal vein on the side of the lesion.

4. Monitoring replacement therapy.

Patients with primary mineralocorticoid deficiency require replacement therapy. Provided the renin control system is intact, measurement of PRA can be used to assess adequacy of treatment.

Patient Preparation

1. Hyper- or hypoaldosteronism.

Please refer to the Aldosterone section, Patient Preparation.

2,3 Renal artery stenosis and location of a renin secreting tumour.

Take blood (5 mL) from left and right renal veins by catheterization.

4. Monitoring replacement therapy.

Take blood (5 mL) at any time of day, after the patient has been established on a particular dose regime.

Sample Preparation

Take a plasma sample: heparin (Leeds, St Mary's) or EDTA (Leeds, St Mary's, UCLH). Use of serum invalidates the assay. Transport to sample to the local laboratory rapidly at room temperature, do not place blood sample in 'fridge' or on ice as renin is enhanced at 4 °C.

The laboratory should separate the plasma promptly, transfer to a fresh plastic container and freeze immediately. Send frozen plasma (1 mL minimum) to the SAS laboratory. Ensure the sample remains frozen during transport. Store remaining sample frozen until the result of the assay is available.

Reference Ranges

For general guidance only: the reference ranges of each SAS centre may vary slightly dependent on methodology.

Adults (20 to 40 years)

(Sodium intake 100 - 150 mmol/day, Potassium intake 50 - 100 mmol/day):

08.00h, after overnight recumbency: 0.5 - 2.2 pmol/mL/h,

08.30h, after 30 min mobility: 1.2 - 4.4 pmol/mL/h.

Random during day: 0.5-3.5 pmol/ml/h

In adults, the baseline PRA and the increment in response to changing from a supine to an upright position decline with advancing age.

According to some authorities, mean values for both these indices after 60 years of age are about half those of young adults.

Infants

The reference ranges for PRA change with age, in the first few weeks of life, values of up to 50 pmol/mL/h have been reported. There is an initial rapid fall, followed by a slower decrease until normal adult levels are reached at about the age of 6 years.

Result Interpretation

1. *Hyper- and hypoaldosteronism.*

The reference ranges above must be considered in association with aldosterone reference ranges.

2. *Renal artery stenosis.*

A high PRA, with a ratio of greater than 1.5:1 in samples from the ischaemic and the contralateral normal kidney, suggests that surgical correction would be successful in reversing the patient's hypertension.

3. *Renin secreting tumour.*

In the absence of chronic renal disease or renal artery stenosis, a greatly elevated PRA in renal vein samples from one side suggests the presence and location of a renin secreting tumour.

4. *Monitoring replacement therapy.*

Plasma renin activity should be compatible with age. Changes in PRA may take 6 weeks to develop in response to changes in the dose of mineralocorticoid.

Quality Assessment

BioRad and sample exchange between centres.

Centres offering this assay

Leeds, London (St Mary's) and London (UCLH).

References

Cartledge S, Lawson N. Aldosterone and renin measurements. *Ann Clin Biochem.* 2000;**37**: 262-78.

Nadar S, Lip GYH, Bevers DG. Primary hyperaldosteronism *Ann Clin Biochem* 2003;**40**:439-52.

McKenna TJ, Sequeira SJ, Heffernan A, Chambers J, Cunningham S (1991) Diagnosis under random conditions of all disorders of the renin-angiotensin-aldosterone axis, including primary aldosteronism. *J Clin Endocrinol Metab* 1991;**73**:952-7

Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension.* 2002;**40**:897-902.

Sayer JA, Pearce SH Diagnosis and clinical biochemistry of inherited tubulopathies. *Ann Clin Biochem.* 2001;**38**:459-70..

Schiemann SJ, Guay-Woodford LM, Thakker RV, Warnock DG Genetic disorders of renal electrolyte transport. *N Eng J Med* 1999; **340**: 1177-87

Stowasser M and Gordon RD. Primary aldosteronism. *Best Practice & Research Clinical Endocrinology & Metabolism* 2003; **17**: 591-605.

Sex Hormone Binding Globulin (SHBG)
(serum)

Somatostatin (plasma)

Clinical Indication

Diagnosis of pancreatic endocrine tumours.

When secreted by endocrine tumours, somatostatin appears to produce symptoms similar to those seen on pharmacological administration i.e. steatorrhoea, diabetes mellitus and gall stones.

Patient Preparation

With the patient at rest after an overnight fast take blood (10mL) into a Lithium Heparin vacutainer or, using a syringe and needle add the blood to a Lithium Heparin tube.

Sample Preparation

Immediately add Trasylol (aprotinin, 0.2mL, 2000 kIU/mL blood). Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result. Send at least 1 mL plasma to the SAS laboratory for somatostatin analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

Reference Ranges

Adults (fasting) <150 pmol/L

Adults with endocrine tumours producing somatostatin >250 pmol/L

Quality Assessment

There are no external quality assessment schemes. There are no other centres offering this assay for sample swapping schemes.

Centre offering this assay

London (Hammersmith).

References

Bloom SR, Long RG. Radioimmunoassay of Gut Regulatory Peptides. London WJ Saunders 1982

Bloom SR, Hammond P. Endocrinology of the Gastrointestinal Tract. In Clinical Endocrinology ed Besser GM and Thorner MO. Wolfe London 1994

Steroid Profile (urine)

Introduction

Urinary steroid profiling by high-resolution gas chromatography (GC) with GC and mass spectrometry (GC-MS) provides a composite picture of the quantitatively major steroid biosynthetic and catabolic pathways. Metabolites of cortisol, progesterone, corticosterone, dehydroepiandrosterone sulphate and testosterone are readily detected whereas those of oestradiol and aldosterone are not. A profile is more informative on adrenocortical than gonadal steroidogenesis and especially useful for investigation of adrenal disorders in newborn infants, taking account of steroid metabolism being markedly different from that of children and adults. A profile overcomes the problems of interference in serum ligand assays from unusual steroids that are present in the neonatal period.

Clinical indications

1. Inborn errors of steroid metabolism.

These include:

(A) Congenital adrenal hyperplasia (CAH) due to deficiency of:

- Pregnenolone synthesis resulting from StAR protein defect in cholesterol uptake or, more rarely, cholesterol side chain cleavage enzyme (CYP11A1) defect (lipoid adrenal hyperplasia)
- 3 β -hydroxysteroid dehydrogenase (HSD3B2)
- 17 α -hydroxylase (CYP 17)
- 11 β -hydroxylase (CYP 11B1)
- 21-hydroxylase (CYP 21A2)
- Cytochrome P450 oxidoreductase (POR, associated with Antley-Bixler syndrome)
- 11-hydroxysteroid dehydrogenase I (HSD 11B1, cortisone reductase, usually apparent, due to an intronic mutation combined with a hexose 6 phosphate dehydrogenase mutation)
- 7-Dehydrocholesterol reductase (DHCR7, associated with Smith Lemli Opitz syndrome, SLOS)
- glucocorticoid receptor action

(B) Deficiency of:

- 5 α -reductase (SRD5A2)
- steroid sulphatase (STS)
- aldosterone synthase (CYP 11B2)
- aromatase (CYP19)
- 11-hydroxysteroid dehydrogenase II (HSD 11B2, apparent mineralocorticoid excess, AME)

2. Steroid-secreting tumours.

3. Steroid resistance.

4. Adrenocortical hyper- and hypofunction.

5. Changed steroid metabolism due to drug treatment (e.g. metyrapone, ketoconazole) or to clinical condition (e.g. Cushing's syndrome, anorexia nervosa)

Clinical uses

1. Virilisation of a newborn female.

This may be the result of androgen excess due to deficiency of:

- 21-hydroxylase
- 11 β -hydroxylase
- 3 β -hydroxysteroid dehydrogenase
- cytochrome P450 oxidoreductase

Characteristic abnormal steroid profiles are found after day 3 of life for each type.

CAH requires life-long treatment and a urinary steroid profile is desirable on any patient with a suspected inborn error of steroid metabolism to define the nature of the steroids in excess.

2. Incomplete virilisation of a male.

A steroid profile is of limited use in the newborn period for the diagnosis of disorders of testosterone production or metabolism. CAH due to StAR or CYP11A1 defects can be inferred and those due to deficiency of 17-hydroxylase, 3 β -hydroxysteroid dehydrogenase and cytochrome P450 oxidoreductase can be detected.

If the boy is older than 3 months a defect of 5 α -reductase is revealed by the finding of a low ratio of 5 α - to 5 β - reduced metabolites of cortisol. In a pubertal child, the defect is also clearly reflected in a low ratio of 5 α - to 5 β - reduced androgen metabolites. 17 β -Hydroxysteroid dehydrogenase deficiency and other causes of low testosterone production are not detected by urine steroid profile analysis.

Incomplete development of the male genitalia in combination with congenital anomalies (microcephaly, mental retardation, hypotonia, short nose with anteverted nostrils, and, in some cases pyloric stenosis) are features of the Smith Lemli Opitz syndrome. This reflects inactivity of 7-dehydrocholesterol reductase. The steroid profile has Δ 7 steroid metabolites. During pregnancy the fetal condition can be detected by the presence of 7-dehydro-oestriol in the urine.

3. Hypertension.

A steroid metabolic cause may be indicated by a low renin level and, not invariably, hypokalaemia. These include:

- 17 α -hydroxylase deficiency. This is only rarely detected in childhood and more usually presents in phenotypic females with delayed puberty, primary infertility, amenorrhoea and hypertension.
- 11 β -hydroxylase deficiency. Hypertension is not obvious in young patients. Girls are virilised at birth, boys usually present with precocious puberty.
- 11 β -hydroxysteroid dehydrogenase II deficiency (apparent mineralocorticoid excess syndrome). This presents with severe hypertension, usually in childhood.
and also:
- Glucocorticoid remediable aldosteronism (GRA). This is characterised by very high concentrations of 18-hydroxycortisol in the urine.

4. Salt-loss.

This may be due to a defect of:

(A) Mineralocorticoid synthesis due to:

- lipoid adrenal hyperplasia
- 3 β -hydroxysteroid dehydrogenase deficiency
- 21-hydroxylase deficiency
- Defects of aldosterone synthase

(B) Mineralocorticoid receptor action (pseudohypoaldosteronism) due to:

- defects in the mineralocorticoid receptor
- defects (more common) in the epithelial sodium channel (ENaC) in the renal tubules.

5. *Hypoglycaemia.*

When present in a newborn it may result from glucocorticoid deficiency due to:

- lipoid adrenal hyperplasia
- 3 β -hydroxysteroid dehydrogenase deficiency
- Congenital adrenal hypoplasia.

6. *Steroid-producing tumours.*

Adrenal tumours may secrete hormones (e.g. cortisol, androgens, 11-deoxycorticosterone), inactive steroids (16 α -hydroxy DHA or pregnenolone) or be non-functional (no steroid production by the tumour). It is useful to have a profile before surgery so that recurrence can be monitored. Gonadal tumours may result in increased plasma androgen and 17-hydroxyprogesterone, and these may be reflected in changes in the urine steroid profile.

7. *Low oestrogens in pregnancy.*

This is most commonly due to steroid sulphatase deficiency. It can also be the result of fetal adrenal hypoplasia and deficiency of aromatase, cytochrome P450 oxidoreductase and 7-dehydrocholesterol reductase. In aromatase deficiency and cytochrome P450 oxidoreductase deficiency the mother may show signs of virilisation from the second trimester.

8. *Congenital adrenal hypoplasia.*

Two types are distinguishable by the urine steroid profile. In the miniature adult type, no fetal zone adrenal steroids are found in urine from newborn infants. In the X-linked cytomegalic type (DAX-1 defect) all steroids are absent or at low levels. Absence of the major steroids may also be due to ACTH deficiency or insensitivity.

9. *Premature adrenarche / precocious puberty / hyperandrogenism in women (polycystic ovary syndrome, PCOS).*

When there are signs of virilisation this may be due to an adrenal or gonadal tumour. Modest hyperandrogenism may be due to a mild form of CAH, most commonly the 21-hydroxylase defect, or to apparent cortisone reductase deficiency. Premature adrenarche and PCOS cannot be defined by profiling but are characterised by high excretion rates of metabolites of cortisol and androgens for age and body size.

10. *Adrenal suppression.*

Steroid metabolites may be suppressed in subjects receiving exogenous glucocorticoids. When synthetic glucocorticoids are suspected in herbal medicines that patients are taking, analysis of cortisol metabolites in a 24h urine collection may be a more productive first approach than direct analysis of the preparation.

11. *Steroid abuse.*

Some synthetic steroid metabolites (e.g. from gestogens, equine oestrogens, androgenic anabolics) can be detected in a urine steroid profile. For a full screen of anabolic steroids, when clinically indicated, we recommend contacting Prof DA Cowan or Dr A Kicman (Dept Forensic Science & Drug Metabolism, King's College, Stamford Street London SE1 9NQ. Tel 020 7848 4848) before sending samples.

Patient Preparation

In cases of ambiguous genitalia it is important to obtain a karyotype. If the patient has hypertension, plasma renin activity and plasma aldosterone concentrations should be checked before considering steroid profile analysis.

For diagnosis of the cause (other than CAH) of salt-loss in a neonate, salt intake and mineralocorticoid treatment should be reduced as much as possible.

Endogenous cortisol production cannot usefully be examined if hydrocortisone or cortisone acetate is being given. If glucocorticoid treatment is essential, dexamethasone is preferred since dexamethasone metabolites do not interfere in the assay. A depot Synacthen test can be used to assess adrenal function during dexamethasone cover.

A 24h urine collection with no preservative is ideal for steroid profile analysis. Untimed collections may be acceptable for the identification of inborn errors of steroid metabolism. Profiles otherwise need to be interpreted with caution because excretion rates of the steroids vary throughout the day, with a peak in the late morning/early afternoon. Expressing steroid excretion as steroid/steroid or steroid/creatinine ratios is potentially misleading.

Sample Preparation

1. Record the 24h urine volume.
2. Transfer 20 mL of urine preferably to a 20 mL Sterilin plastic bottle with plastic lid. Do not overfill the bottle and stand it upright if freezing prior to dispatch.
3. Do not use Parafilm on the inside of the lid.
4. Record on the SAS request form:
 - the 24 h volume or duration of the collection
 - age and sex of the patient
 - clinical details and any relevant treatment.

Quality Assessment

UKNEQAS / ERNDIM

Reference ranges

The SAS Laboratory will provide appropriate reference data and an interpretation of results based on relevant biochemical and clinical information.

Centres offering the assay

London (Kings), London (UCL).

References

Honour JW. "Steroid profiling". *Annals of Clinical Biochemistry* 1997; 34: 32-44.

Honour JW and Brook CGD. "Clinical indications for the use of urinary steroid profiles in neonates and children." *Annals of Clinical Biochemistry*. 1997; 34: 45-54.

Anomalous thyroid function tests

(serum)

Introduction

The measurement of free thyroid hormones and TSH by immunoassay can still be problematic. Discrepancies may be found between different assays for free thyroxine or there may be inconsistencies between TSH and free thyroxine as measured by routine assays and the clinical picture eg raised free T4 with TSH within the reference range. Before rare causes of this biochemical picture such as TSH secreting pituitary tumours and thyroid hormones resistance are investigated, analytical interference should be excluded. Such interferences would include antibodies to TSH, T4 or T3, familial dysalbuminaemic hyperthyroxinaemia (FDH) and heterophilic antibodies. Measurement of free T4 by equilibrium dialysis, total T4 or linearity of dilution in a two-step assay can also be used to confirm an elevated free T4. PEG precipitation can be used as a screen for antibody interference with TSH.

Clinical Indications

1. Investigation of anomalous thyroid function test results
2. Investigation of rare clinical conditions of thyroid and pituitary disease.
3. Investigation of familial dysalbuminaemic hyperthyroxinaemia

Method Information

Delfia TSH assay (Cambridge)

Delfia Free T4 two-step assay (Cambridge)

Delfia Total thyroxine (Cambridge)

Equilibrium dialysis free T4 (Birmingham)

Screen test for antibodies to T4 and T3 (Birmingham)

Screen test for antibody interference in TSH assay (Cambridge)

Screen test for FDH (Birmingham)

Screening test for heterophilic antibodies (assay specific) (Birmingham, Cambridge)

Patient Preparation

None.

Avoid collection when patient heparinised.

Exclude treatment with amiodarone or heparin as a cause of anomalous TFT results.

Please include results of thyroid function tests and methods used and full clinical details especially treatment with thyroid hormones or anti-thyroid medication.

Sample Preparation

Plain tube (serum).

Volume required is dependant on assays required. Preliminary investigations can be made with 2 mL serum. Store frozen at – 20 °C. Transport frozen is necessary if samples are being sent from outside the UK.

Result Interpretation

Birmingham:

Screening tests are reported as either positive or negative with an appropriate comment.

Cambridge:

Two-step free T4 delfia assay with dilution test is reported.

Delfia TSH with % recovery after PEG precipitation.

Total T4

Other tests can be referred as necessary, sample volume permitting (Birmingham tests, Total T3, SHBG, α subunit, THR β genotyping, if whole blood EDTA sample provided)

Quality Assessment

Equilibrium dialysis free T4 UKNEQAS

Total T4 UKNEQAS

The two SAS centres exchange samples on a 'case for investigation' basis. No EQA scheme is available for the other tests.

Centres offering these assays

Birmingham:

Screen for antibodies to T4 and T3

Screen for heterophilic antibodies

Screen for familial dysalbuminaemic hyperthyroxinaemia

Equilibrium dialysis free T4

Cambridge:

Two-step free T4 assay with dilution test

Total T4

Deflia TSH with PEG recovery

Screen for anti-animal antibodies

References

Nelson JC, Tomei RT. Direct determination of free thyroxine in undiluted serum by equilibrium dialysis/radioimmunoassay. *Clin Chem* 1988; **34**: 1737-44.

Sakata S, Nakamura S, Miiura K. Autoantibodies against thyroid hormones or iodothyronine. *Ann Int Med* 1985; **103**: 579-89.

Sunthornthepvarakul T, Likitmaskul S, Ngowngarmratana S, Angsusingha K, Kitvitayasak S, Scherberg NH, Refetoff S. Familial Dysalbuminemic Hypertriiodothyroninemia: A New, Dominantly Inherited Albumin Defect. *J Clin Endocrinol Metab* 1998; **83**: 1448-54.

Thyroid Hormone Receptor β Gene Sequence Analysis (EDTA whole blood)

Introduction

Resistance to Thyroid Hormone (RTH)

The syndrome of resistance to thyroid hormone is characterized by elevated circulating FT4 and FT3 concentrations, failure to suppress pituitary TSH secretion and variable peripheral tissue refractoriness to hormone action. The disorder is inherited in an autosomal dominant manner in 75% of families but sporadic cases are described (15-20%). RTH is associated with diverse, heterozygous, loss-of-function mutations in the thyroid hormone β receptor gene. Patients with RTH can present in two ways: some individuals are asymptomatic and identified when thyroid function tests (TFTs) are undertaken for other reasons; in other cases, patients may experience thyrotoxic symptoms (failure to thrive in childhood; anxiety, tachycardia, weight loss) which prompt thyroid investigation. These patients may present with hyperthyroxinaemia and a non-suppressed TSH.

Interference with thyroid hormone and TSH measurement (e.g. antiiodothyronine or antiTSH antibodies) is much commoner than resistance to thyroid hormone or pituitary tumours secreting TSH (TSHoma), so it is advisable to first exclude analytical interference and pituitary disease. For the investigation of these see the sections on (1) Alpha Subunit, (2) Equilibrium dialysis free T4 and (3) Anomalous thyroid function tests.

Other investigations: Normal concentrations of serum sex hormone binding globulin (SHBG), a hepatic marker of thyroid hormone action, or abnormal TFTs in first degree relatives favours a diagnosis of RTH. In ~15% of RTH cases, an abnormality in the TR β gene cannot be identified, raising the possibility that defects in other genes mediating thyroid hormone action could result in a similar biochemical picture.

Clinical Indication

Suspected Resistance to Thyroid Hormone (RTH).

Method Information

DNA sequencing using fluorescent di-deoxy terminators. Genomic DNA is extracted from EDTA blood and DNA encoding exons V-VIII of the THR β Gene including splice sites are PCR amplified prior to sequencing.

Quality Assessment

EMQN <http://www.emqn.org/emqn/>

Patient Preparation

Thyroid hormone receptor gene sequence analysis is a genetic test. Before taking any blood sample it is essential that the patient receives appropriate counselling. It is the responsibility of the clinician caring for the patient to ensure that this is done and samples are only accepted on the basis that patients have been counselled and consent to the analysis.

Sample Handling

Before requesting sequencing, it is strongly recommended that other causes of hyperthyroxinaemia with non-suppressed TSH have been excluded. Provision of a summary of the clinical background of the case is valuable. Please include results of thyroid function tests and other relevant investigations (eg SHBG, α -subunit) undertaken locally and any medication which could influence TFTs.

Send 5 mL of whole blood in EDTA tubes to the SAS laboratory by first class post. If 5ml of serum is provided common assay interference will be screened for prior to sequencing.

Interpretation

Please contact the SAS laboratory.

Centre offering this assay

Cambridge.

References

Chatterjee VKK.& Gurnell M. Resistance to thyroid hormone in Oxford Textbook of Endocrinology and Diabetes (Eds JAH Wass and S.M.Shalet) Oxford University Press, 2002

Thyrotrophin receptor (TSH-R) gain of function mutations

(Whole blood EDTA)

Introduction

The TSH-R is a G protein coupled receptor that mediates the effect of TSH in thyroid development, growth and synthetic function. Thyroid cell proliferation and thyrotoxicosis can occur as a result of gain of function mutations in the TSH-R. Germline mutations cause familial or sporadic hyperthyroidism and are inherited in an autosomal dominant fashion requiring only one allele to be affected to cause hyperthyroidism. They are almost exclusively confined to exon 10 of the TSH-R, which encodes the membrane spanning region. A gain of function mutation has also been identified in exon 9.

Graves' disease, toxic nodules and toxic multinodular goitres are the commonest causes of hyperthyroidism. However, in cases where the cause of hyperthyroidism is not clear, identification of germline mutations in the TSH-R may be useful. For patients with thyrotoxicosis due to gain of function TSH-R mutations thyroidectomy is the only absolute treatment. Mutation identification in family members is also useful, and may allow identification of affected family members before clinical presentation¹. It is however important to note that familial hyperthyroidism due to TSH-R gain of function mutations is a relatively rare cause of hyperthyroidism and that before considering TSH-R sequencing more common causes of hyperthyroidism should be excluded.

Hyperthyroidism during pregnancy may be associated with Graves' disease, due to stimulation of the TSH-R by TSH-R antibodies, or with hyperemesis gravidarum due to stimulation of the TSH-R by hCG. There has also been one case report of a polymorphism in exon 7 of the TSH-R which confers increased responsiveness to hCG and results in hyperthyroidism when serum concentrations of HCG are increased².

Clinical Indication

TSH-R sequencing is likely to be useful to paediatricians investigating hyperthyroid children. Pointers that a TSH-R gain of function may be the cause include: negative TRAb when the patient is hyperthyroid, goitre, +/- eye signs (there is debate about this point), +/- a family history of hyperthyroidism. Positive aTPO status should not discourage TSH-R sequencing as the presence of aTPO (but not TRAb) has been described in one patient with a TSH-R gain of function mutation¹.

TSH-R sequencing may also be of use in the investigation of hyperthyroidism during pregnancy which is not associated with Graves' disease, toxic adenomas or multinodular goitres but which requires treatment throughout the duration of the pregnancy.

Method Information TSH-R gene sequencing

Graves' disease as a cause of hyperthyroidism should be excluded by measurement of TRAb prior to proceeding to TSH-R sequencing. Since all described gain of function mutations have been found in exons 10 and 9, sequencing will be restricted to these exons. A database of previously characterised mutations is available³. Novel mutations will require characterisation using functional studies.

If a polymorphism in the TSH-R which confers increased sensitivity to hCG is suspected, exon 7, followed by the remaining exons which comprise the extra-cellular domain of the receptor (1-6+ 8-9 + part of 10) will be sequenced.

Patient Preparation

Thyroid hormone receptor gene sequence analysis is a genetic test. Before taking any blood sample it is essential that the patient receives appropriate counselling. It is the responsibility of the clinician caring for the patient to ensure that this is done and samples are only accepted on the basis that patients have been counselled and consent to the analysis.

Sample Handling

Whole blood (5 mL EDTA), transported by first class post.

Result Interpretation

Please contact Dr Marian Ludgate (Senior lecturer, Centre for Endocrine & Diabetes Sciences, School of Medicine, Cardiff University, Heath Park, Cardiff) and Dr Carol Evans.

Quality Assessment

None available.

Centre offering this assay

Cardiff.

References

1. Fuhrer D. Warner J. Sequeira M. Paschke R. Gregory J. Ludgate M. Novel TSHR germline mutation (Met463Val) masquerading as Graves' disease in a large Welsh kindred with hyperthyroidism. *Thyroid* 2000; **10(12)**:1035-41.
2. Rodien P., Bremont C., Sanson M.L. et al., Familial gestational hyperthyroidism caused by a mutant thyrotrophin receptor hypersensitive to human chorionic gonadotrophin. *New England Journal of Medicine* 1998; **339 (25)**: 1823-6.
3. www.uni-leipzig.de/innere/tsh/

Thyrotrophin receptor autoantibodies (TRAb)

(serum)

(formerly known as TBII – Thyroid Binding Inhibiting Immunoglobulin)

Introduction

Graves' disease is the commonest cause of hyperthyroidism in the UK and is caused by antibodies that bind and stimulate the thyrotrophin receptor (TSH-R). In general, the diagnosis of Graves' disease is made on the clinical presentation of the patient (e.g. hyperthyroidism, goitre +/- thyroid eye disease and or pretibial myxoedema). But where there is doubt, the diagnosis can be made by measurement of TSH-R antibodies (TRAb). It is important to note that Graves' disease is characterised by periods of disease exacerbation as well as remission which coincide with the appearance and disappearance of TRAb in patients serum. Measurement of TRAb is therefore a more sensitive marker of Graves' disease when the patient is hyperthyroid. Absence of TRAb when the patient is euthyroid does not necessarily exclude Graves' disease.

If Graves' disease occurs in pregnancy, placental transfer of TSH-R stimulating antibodies from a mother to her foetus results in transient neonatal hyperthyroidism. Early diagnosis and treatment of pregnant women with Graves' disease is important to prevent complications in the foetus and newborn.

TRAb which block thyroid activation are also found in the serum of patients with autoimmune hypothyroidism, and cause hypothyroidism. During pregnancy, passage of maternal TSH-R blocking antibodies (TBAb) across the placenta can cause transient hypothyroidism in the neonate. Transient congenital hypothyroidism due to TBAb generally occurs in infants of mothers with a history of autoimmune hypothyroidism who are taking thyroxine or have undiagnosed hypothyroidism. TBAb occur more frequently in Asian than in Caucasian populations. In the West, TBAb is estimated to account for 1-2% of cases of congenital hypothyroidism^{1,2}. TBAb are found in both maternal and infant serum at birth but gradually clear from the infant's circulation after 3-4 months.

Clinical Indication

Measurement of TRAb may be useful in several clinical situations^{3,4,5,6}

1. To confirm a diagnosis of Graves' disease.
2. To diagnose or predict transient neonatal hyperthyroidism due to transplacental passage of TSH-R stimulating antibodies during pregnancy. Measurement of TRAb is useful in early pregnancy for women who have had prior radioiodine therapy or thyroidectomy for hyperthyroidism in whom the presence of TRAb will be clinically silent and in late pregnancy in active Graves's.
3. To diagnose transient neonatal hypothyroidism due to transplacental passage of TSH-R blocking antibodies during pregnancy.
4. For the diagnosis of thyroid associated ophthalmopathy.

Method Information

TRAb is measured using a competitive binding assay which relies on the ability of TSH-R antibodies to displace radiolabelled TSH from solubilised human or porcine recombinant TSH-R. The TRAb assay does not distinguish between TSH-R stimulating and TSH-R blocking antibodies, but this can generally be determined from the clinical presentation of the patient.

Patient Preparation

None. Take blood (5 mL into a syringe or red topped vacutainer)

Sample Handling

Serum (minimum 0.5 mL), transported to the SAS centre by first class post. Assays are performed weekly or fortnightly, depending on demand (average turnaround 8 days), although additional assays can be arranged to accommodate urgent requests.

Reference range

TRAb < 1IU/L regarded as negative, TRAb > 1.5 IU/L as positive⁷.

Quality Assessment

There is no external quality assessment scheme for this assay but the three laboratories participate in a regular sample sharing scheme and jointly review the results obtained.

Centres offering this assay

Cambridge, Cardiff, Newcastle.

References

1. Brown RS, Bellisario RL, Botero D, Fournier L, Abrams CA, Cowger ML, David R, Fort P, Richman RA Incidence of Transient Congenital Hypothyroidism due to Maternal Thyrotrophin Receptor-Blocking Antibodies in Over One Million Babies. *J Clin Endocrinol Metab* 1996; **81**: 1147-1151.
2. Orgiazzi J Anti-TSH receptor antibodies in clinical practice. *Endocrinol Metab Clin North Am* 2000; **200**: 339-355.
3. Schott M, Scherbaum WA, Morgenthaler NG. Thyrotropin receptor autoantibodies in Graves' disease. [Review] *Trends in Endocrinology & Metabolism* 2005; *16*(5): 243-8.
4. Laurberg P., Nygaard B., Glinoe D., Grussendorf M., Orgiazzi J. Guidelines for TSH-receptor antibody measurement in pregnancy: results of an evidence-based symposium organised by the European Thyroid Association. *Eur J Endocrinol* 1998; **139**:584-586.
5. McKenzie J, Zakarija M. Fetal and Neonatal Hyperthyroidism and Hypothyroidism due to Maternal TSH Receptor Antibodies. *Thyroid* 1992; **2**: 155-159.
6. Burman, K. & Pandian, R. Clinical Utility of TSH receptor antibodies. *The Endocrinologist* 1998; **8**: 284-290.
7. Costagliola S et al. Second generation assay for TSH-receptor antibodies has superior diagnostic sensitivity for Grave's disease. *J Clin Endocrinol Metab.* 1999; **84** (1): 90-97.

Thyroglobulin (serum)

Introduction

Thyroglobulin is a large molecular weight protein synthesized in the thyroid and may thus be used as a marker for differentiated thyroid cancer.

Clinical Indication

Evaluation and management of patients treated for differentiated thyroid cancer with surgery and radioiodine, and receiving thyroid hormone therapy.

Measurement of thyroglobulin is of no value in the initial diagnosis of thyroid cancer. After ablative surgery, thyroglobulin levels do not discriminate between the presence of normal thyroid tissue and tumour unless thyroid hormone replacement is given. Serial measurements in such patients aid in monitoring for possible recurrence.

Endogenous antibodies to thyroglobulin may cause either negative or positive interference in the assay, which is difficult to predict. Measurement of thyroglobulin antibodies, recovery experiments and discrepancy between radioimmunoassay and immunometric assay results may indicate interference.

Method Information

Immunometric (Cardiff, Newcastle)

Radioimmunoassay (Birmingham)

Patient Preparation

Samples may be collected when a patient is on suppressive doses of thyroxine, when the TSH is elevated or post recombinant TSH (Thyrogen), depending on the requesting centres clinical protocols.

Samples should not be collected immediately post radioiodine treatment or imaging, nor post-fine needle aspiration of the thyroid.

No patient preparation is required. Take blood (5 mL) into a syringe or red topped vacutainer.

Sample Handling

Transfer the blood to a plain tube. Allow to clot at room temperature, avoiding haemolysis which, with lipaemia, can produce spurious results. Send serum (2 mL) to the SAS laboratory by first class post.

Interpretation

A reference range obtained in a euthyroid population is not applicable to a patient population post-thyroid surgery

Please contact the SAS laboratory.

Quality Assessment

UK NEQAS.

Centres offering this assay

Birmingham, Cardiff, Newcastle.

References

Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti, JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2005; **90**:5566-5575.

Stockigt JS. Editorial: ambiguous thyroglobulin assay results in the follow-up of differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2005; **90**: 5904-5.

Guidelines for the management of thyroid cancer in adults. British Thyroid Association and Royal college of Physicians. 2002.

UK Guidelines for Thyroid Function tests. Association of Clinical Biochemists, British Thyroid Association, British Thyroid Foundation. Draft for Open Consultation October 2005.

Clark PM, Beckett G. Can we measure serum thyroglobulin? *Ann Clin Biochem* 2002; **39**:196-202.

Vasoactive Intestinal Peptide (VIP) (plasma)

Clinical Indication

Diagnosis of pancreatic tumour or a ganglioneuroma which secretes vasoactive intestinal peptide (VIP).

Chronic profuse watery diarrhoea is the commonest indication for this test. Administration of VIP to animals causes hyperglycaemia, inhibition of gastric acid, secretion of pancreatic bicarbonate and of small intestine juice and lowering of systemic blood pressure with skin flush. These features may also be seen in patients with a tumour which is secreting VIP.

Patient Preparation

With the patient at rest after an overnight fast take blood (10 mL) into a Lithium Heparin vacutainer or, using a syringe and needle add the blood to a Lithium Heparin tube.

Sample Preparation

Immediately add Trasylol (aprotonin, 0.2mL, 2000 kIU/mL blood). Mix by inversion, place on ice and transfer to the laboratory. Separate the plasma in a refrigerated centrifuge at approximately 4°C and store the plasma at -20°C. The plasma must be frozen within 30 minutes of venepuncture. Visible haemolysis invalidates the result. Send at least 1 mL plasma to the SAS laboratory for VIP analysis. If the request is part of a full gut hormone screen an absolute minimum of 3 mL of plasma must be sent. Ensure the sample remains frozen during transport.

Reference Ranges

Adults (fasting) <30 pmol/L

Adults with endocrine tumours producing VIP >80 pmol/L

Quality Assessment

There are no external quality assessment schemes. There are no other centres offering this assay for sample swapping schemes.

Centre offering this assay

London (Hammersmith).

References

Bloom SR, Long RG. Radioimmunoassay of Gut Regulatory Peptides. London WJ Saunders 1982

Bloom SR, Hammond P. Endocrinology of the Gastrointestinal Tract. In Clinical Endocrinology ed Besser GM and Thorner MO. Wolfe London 1994