Endocrine Dynamic Function Test Protocols - ADULTS

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THYROID

Insulin Tolerance Test

This test is the gold standard for assessing the integrity of the hypothalamo-pituitary-adrenal axis. Reproducibility among healthy volunteers is well documented but not known amongst patients with pituitary disease.

ACTH and GH are both released as part of the stress mechanism triggered by insulin induced hypoglycaemia.

Indications

1. Diagnosis of secondary adrenal failure.
2. Diagnosis of growth hormone deficiency.
3. Differentiation of Cushing's Syndrome from pseudo-Cushing's eg. Depression, Alcohol excess

Contraindications

- **Not to be used in children <16 years, refer to the Paediatric DFT protocols**
- Age >60 years
- Ischaemic Heart Disease
- Epilepsy or unexplained blackouts
- Severe panhypopituitarism, hypoadrenalism (9am cortisol <100nmol/L)
- Untreated hypothyroidism (impairs the GH and cortisol response),
- Glycogen Storage Disease
- Hypocalcaemia/Hypokalaemia

Preparations and precautions

- Patient should fast from midnight (water permitted) and be recumbent during the test.
- ECG must be normal and the patient's weight known
- Serum Cortisol must be >100nmol/L at 9am
- If patient is taking hydrocortisone the morning dose should be omitted
- For non-urgent cases, combined OCP and HRT should be stopped for 6 weeks prior to the test.
- Intravenous dextrose and intravenous hydrocortisone should be readily available

Side effects

- Sweating
- Palpitation
- Loss of consciousness
- convulsions due to severe hypoglycaemia (rare)

Requirements:

- Soluble insulin (Actrapid):
  - 0.15U/kg for normal subjects
  - 0.10U/kg for hypopituitary subjects
  - 0.2-0.3U/kg for subjects with acromegaly, diabetes or Cushing's syndrome
- If symptomatic or biochemical hypoglycaemia is not achieved after 30 mins, consider giving additional half dose of insulin – discuss with endocrinology medical staff. This may be required for acromegalic and diabetic patients.
- 10% dextrose (250ml) or glucagon 1mg available for immediate administration for hypoglycaemia.
- 100mg ampoule of hydrocortisone, 0.9% saline available for immediate administration for hypoadrenal crisis
- Orange juice or Gluco juice
- Indwelling cannula, 3 way tap.
- 6 brown top serum tubes
- 6 yellow fluoride EDTA tubes

Procedure

PATIENT PREPARATION

- Patient should fast from midnight (water permitted) and be recumbent during the test.
- Perform a 9 am serum cortisol
- Result must be reviewed by a doctor
- If the patient is hypoadrenal for any reason (9am cortisol <100nmol/L) (or on hydrocortisone or prednisolone), the case must be discussed with senior medical staff before administration of insulin.
- Perform an ECG (which must be normal to proceed)
- Weigh the patient and document accurately, this is required to calculate the insulin dose required.

TEST

This test is potentially dangerous.
A doctor or nurse must be in attendance at all times.
Reverse hypoglycaemia with oral orange juice 150mls or Gluco juice (entire bottle), see Trust policy on treatment of hypoglycaemia in adults.
If, during the test, the patient shows severe symptoms/ signs of hypoglycaemia (drowsiness, incipient/actual loss of consciousness or fits) then terminate the test with 250ml 10% dextrose IV or 1mg Glucagon IM/IV/SC.
If feasible continue with blood sampling as adequate pituitary stimulation will have occurred.

Minutes	Procedure	Samples
-30	Insert iv cannula	
0	Take basal blood samples Inject soluble insulin as an iv bolus	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
30	Take samples for **GH, cortisol and glucose** Observe symptoms and record Take a glucometer strip reading	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
60	Take samples for **GH, cortisol and glucose** Observe symptoms and record Take a glucometer strip reading	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
90	Take samples for **GH, cortisol and glucose** Observe symptoms and record Take a glucometer strip reading	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
120	Take samples for **GH, cortisol and glucose** Observe symptoms and record Take a glucometer strip reading	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)

If patient has a hypoadrenal crisis they should receive IV hydrocortisone 100mg and IV 0.9% saline (250-500ml or more based on clinical assessment)

Aftercare

Upon completion of test give patient a supervised meal and observe for 2 hours.
Ensure glucose is normal before discharging using glucometer.
Explain to the patient the need to eat well, to avoid strenuous exercise and to avoid driving for the rest of the day. Give the patient the aftercare advice leaflet.

Interpretation of results

The test can not be interpreted unless hypoglycaemia (**glucose < 2.2 mmol/L measured by the laboratory**) has been achieved or the patient has shown good evidence of symptomatic hypoglycaemia.

Normal Response

Glucose	<2.2mmol/L measured by the laboratory
Cortisol	Peak > 430nmol/L
Growth Hormone	Peak >6.7 µg/L

Growth hormone deficiency of sufficient severity for GH replacement to be of benefit, is present in adults whose peak GH is <3 µg/L.
The European and Endo Society guidelines and NICE define severe GHD as GH <3 µg/L and partial <5 µg/L

An inadequate GH response may occur in obese patients, and those who have had a recent spontaneous pulse of GH (high GH level at zero sample).

Insulin Tolerance Test
Blood Glucose/Hypoglycaemia Chart

Name: **Hosp No:**

Diagnosis: **ECG: Normal/Abnormal**

Wt in Kg:

Dose of Insulin given:

TIME (mins)	Test Strip Glucose mmol/L	Symptoms
0		
30		
60		
90		
120		
150		

Symptoms experienced during ITT
Tick all that apply

Sweating **Drowsiness**
Tremor **Confusion**
Tachycardia **In coordination**
Hunger **Slurred Speech**
Malaise **Strange behaviour**
Headache **Seizure(s)**

Extra insulin given? YES/NO

Dose :

Glucagon Stimulation Test

Glucagon increases blood glucose which causes insulin release and indirectly stimulates GH and ACTH release through provocation of the hypothalamic-pituitary axis.

Indications
Assessment of growth hormone and ACTH/cortisol reserve especially when insulin-induced hypoglycaemia is contra-indicated.

Contraindications
- Phaeochromocytoma or insulinoma (may provoke an attack)
- Starvation >48 hours or glycogen storage diseases (inability to mobilise glycogen may result in hypoglycaemia)
- Severe hypocortisolaemia (09:00 cortisol <100 nmol/L)
- Thyroxine deficiency may reduce GH and cortisol response
- This test is unreliable in patients with Diabetes Mellitus

Side Effects
Glucagon may cause nausea, vomiting and abdominal pain

Requirements
- 6 yellow top fluoride EDTA tubes
- 6 brown top serum tubes

Procedure

PATIENT PREPARATION

- Systemic steroids prednisolone and dexamethasone should be stopped 24 hours before the test
- If patient is taking hydrocortisone the morning dose should be omitted
- For non-urgent cases, oral oestrogens (combined OCP and HRT) should be stopped for 6 weeks prior to the test, (transdermal oestrogens can be continued)
- Patient should fast from midnight (water permitted) and be recumbent during the test.
- Perform a 9 am serum cortisol. Result should be reviewed by a doctor. If the patient is hypoadrenal for any reason (9am cortisol <100nmol/L) the case must be discussed with senior medical staff before administering glucagon
- Calculate glucagon dose: adults: 1 mg, (1.5mg if >90kg)

TEST

Minutes	Procedure	Samples
-30	Insert an indwelling cannula	
0	Take basal samples for **glucose, cortisol and GH**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
0	Give the glucagon **im**	
90	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
120	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
150	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
180	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
210	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)
240	Take samples for **glucose, cortisol and GH.**	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)

Interpretation of results

Normal response

Cortisol Peak: >430nmol/L
Growth Hormone Peak: >6.7 µg/L.
Glucose Should show a transient fall followed by a rise.
The European and Endo Society guidelines and NICE define severe GHD as GH <3 µg/L and partial <5 µg/L

SENSITIVITY AND SPECIFICITY

This is a less reliable test of somatotroph and corticotroph function than the ITT. It is an excellent alternative in patients who can not tolerate hypoglycaemia because of epilepsy, ischaemic heart disease or hypopituitarism. The false negative rate for cortisol response is 30%. with a sensitivity of 71% and a specificity of 57% for adequate cortisol reserve if a peak cortisol cut-off of >350 nmol/L is used. Only 4-8 % of normals will not show an adequate rise in GH: this is usually in patients over 50.

Short Synacthen Test

Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the anterior pituitary. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactide; Synacthen ®). It does not test the whole pituitary-adrenal axis.

Indications

1. Used in the diagnosis of hypoadrenalism as a screening test.
2. It is an increasingly used alternative to the insulin tolerance test to diagnose secondary hypoadrenalism due to pituitary hypofunction. However, it should not be used in the early post-operative assessment of the hypothalamic-pituitary-adrenal axis as response may be normal (an insulin tolerance/glucagon stress test should be used instead).
3. May also be used to ascertain that the adrenals are functioning normally after a prolonged course of corticosteroids, or after suppression by Cushing's syndrome (e.g. after removal of a unilateral Cushing's adrenal adenoma).

Contraindications

Test not required for assessment of hypoadrenalism if random cortisol >430 nmol/L.
The Short Synacthen test gives unreliable results within 2 weeks of pituitary surgery.

Preparations and precautions

- Glucocorticoid replacement on the day of the test invalidates the test.
- Prednisolone should be stopped 24 hours before the Short Synacthen test.
- Hydrocortisone should be omitted on the morning of the Short Synacthen test.
- For non-urgent cases, combined OCP and HRT should be stopped for 6 weeks prior to the test. Pregnancy will also affect results due to the increase in CBG.

Side Effects

- There are rare reports of hypersensitivity reactions to Synacthen particularly in patients with history of allergic disorders. Adrenal haemorrhage has also been reported rarely.

Requirements

- 250 µg Synacthen (1 ampoule)
- 2x brown top serum tubes
- 1 x pink EDTA tube

Procedure

- This test should be performed preferably in the morning between 0800 and 0900 hours but can be performed later in the day.

Minutes	Procedure	Sample
0	Take 3ml blood for Cortisol and 3ml for ACTH then administer 250µg Synacthen IM / IV	1 x brown top serum (cortisol) 1 x pink EDTA (ACTH)
30	Take 3ml blood for Cortisol	1 x brown top serum (cortisol)

N.B A 60 minute sample may be taken when assessing adrenal reserve post steroid therapy
For patients on prednisolone, particularly respiratory patients, cortisol should be measured by mass spectrometry due to interference in immunoassay. This can be requested in EPIC once patient is identified as being on prednisolone.

Interpretation of results

Adrenal insufficiency is excluded by a 30 min value >430 nmol/L.

If impaired cortisol response, and ACTH >200 ng/L the diagnosis is primary adrenal failure.

If ACTH <10ng/L then diagnosis is secondary adrenal failure.

Serum cortisol >650 nmol/L excludes deficiency in patients on oestrogens. [Reference El-Farahan Clin Endo (2013) **78** 673-80.

For patients on glucocorticoid replacement interpretation of the response is not straightforward and depends on the duration, and dose of glucocorticoid treatment received.

Synacthen Stimulation of 17αOHP

Adrenal glucocorticoid secretion is controlled by adrenocorticotrophic hormone (ACTH) released by the anterior pituitary. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactide: Synacthen ®). In subjects with enzyme deficiency in the steroid synthetic pathway, cortisol may, or may not, be adequately secreted. However, there is excessive secretion of the precursor steroids before the defective enzyme. The commonest form of CAH is due to deficiency of 21-hydroxylase and in these subjects increased secretion of 17αOH-progesterone (17 OHP) can be detected.

Indications
This is performed for the investigation of congenital adrenal hyperplasia (CAH).

Contraindications
The Synacthen test gives unreliable results within 2 weeks of pituitary surgery.

Side Effects
There are rare reports of hypersensitivity reactions to Synacthen particularly in patients with a history of allergic disorders.

Preparations and precautions
- Prednisolone should be stopped 24 hours before the Short Synacthen test.
- Hydrocortisone should be omitted on the morning of the Short Synacthen test.
- Consider sending a random urine for urine steroid profile (10mL Monovette urine tube)

Requirements
- 6 x brown top serum tubes
- 250 microgram Synacthen (1 ampoule)

Procedure
- This test should be performed preferably in the morning between 0800 and 0900 hours but can be performed later in the day.

Minutes	Procedure	Sample
0	Take sample for **Cortisol and 17 OHP** and then administer 250µg Synacthen IV / IM	2 x brown top serum tube (cortisol and 17OHP)
30	Take sample for **Cortisol and 17 OHP**	2 x brown top serum tube (cortisol and 17OHP)
60	Take sample for **Cortisol and 17 OHP**	2 x brown top serum tube (cortisol and 17OHP)

Interpretation of results

Cortisol normal response at 30 mins: Peak > 430nmol/L

17-OHP Unaffected adults and children usually have a basal 17-OHP of <6 nmol/L.

A minority of patients with non-classical CAH have a normal basal 17-OHP, even on early morning samples.

A normal response to Synacthen is a stimulated 17-OHP of <9.8 nmol/L at 60 minutes.

A stimulated 17-OHP between ≥9.8 but ≤30 nmol/L is an equivocal response and CAH is not excluded. Genotyping and/or a urine steroid profile is recommended.

A stimulated 17-OHP of ≥30 nmol/L is consistent with a diagnosis of CAH. Genotyping of the 21-hydroxylase gene and urine steroid profiling can be used to confirm the diagnosis.

Milder elevations of 17-OHP may be found in rarer forms of CAH: 11-β-hydroxylase deficiency and 3-β-hydroxysteroid dehydrogenase deficiency.

Saline Infusion Test for Hyperaldosteronism

The principle of this test is that in hyperaldosteronism, control of aldosterone secretion is lost and is not suppressed in response to an excessive salt and water load.

Indications
This test is a second line test for the diagnosis of primary aldosteronism.

Patients should already have been screened with a random Aldosterone:Renin Ratio (ARR) > 1000

Contraindications

This test should not be performed in patients with any of the following

- severe uncontrolled hypertension
- renal insufficiency
- cardiac insufficiency
- cardiac arrhythmia
- severe hypokalaemia

Requirements
- 2L 0.9% saline for IV administration
- infusion pump/giving set
- 2 indwelling catheters
- 2 pink top EDTA for plasma renin and aldosterone
- 2 brown top serum tubes
- Blood samples should be taken immediately (within 30 minutes) to the laboratory but not on ice as PRA (plasma renin activity) is measured by the activity of renin and at 4^{o}C the inactive renin precursor is maximally converted to active renin.

Procedure

PATIENT PREPARATION

- Stop mineralocorticoid receptor antagonists (spironolactone and eplerenone) for 6 weeks before the test
- Stop diuretics 4 weeks before the test.
- Stop beta blockers, calcium channel antagonists, ACE inhibitors and AT2 blockers for 2 weeks before the test.
- Can continue to use alpha blockers to manage hypertension. Alternative antihypertensives that can be taken are doxazosin, slow release verapamil, hydralazine with slow release verapamil (to avoid reflex tachycardia)
- Ensure plasma K in normal range (ideally >4) prior to performing test
- Examine patient for signs of cardiac failure. Check BP.

TEST

- Position patient in seated position for at least 30 minutes prior to commencing procedure and sampling. The patient should remain seated throughout test.
- Blood pressure, oxygen saturation and heart rate are monitored hourly throughout the test.

Time (Minutes)	Procedure	Samples
-15	Site indwelling cannula for administration of 0.9% Saline infusion and cannula in opposite arm for blood sampling and leave for 30 mins	
0	Take sample for Aldosterone, Plasma renin activity and U and E's	1 x pink top EDTA tube (renin and aldosterone) **NOT ON ICE** **NB. send sample immediately as must be received in laboratory within 30mins** 1 x brown top serum (U and E's)
0	Commence Infusion of 2L 0.9% saline over 4 hours	
240	STOP INFUSION	
240	Take sample for Aldosterone, Plasma renin activity and U and E's	1 x pink top EDTA tube (renin and aldosterone) **NOT ON ICE** **NB. send sample immediately as must be received in laboratory within 30mins** 1 x brown top serum (U and E's)

Interpretation of results[1]

The lack of suppression of aldosterone excretion with intravascular expansion indicates primary hyperaldosteronism.

Post-infusion plasma aldosterone <162 pmol/L makes the diagnosis of primary hyperaldosteronism unlikely. Aldosterone ≥ 162 pmol/L Consistent with primary hyperaldosteronism, further investigation warranted.

References

[1] Comparison of seated with recumbent saline suppression testing for the diagnosis of primary aldosteronism. Stowasser M et al. JCEM 2018; 103:4 113-4124

[2] Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline. JCEM 2016

Captopril challenge test for hyperaldosteronism

The principle of the test is that normal individuals suppress aldosterone completely after captopril, an ACE inhibitor. In hyperaldosteronism there is a failure to suppress aldosterone.

Indications
This test is a second line test for the diagnosis of primary aldosteronism. It has lower sensitivity than the saline infusion test (SIT) for hyperaldosteronism, however can be considered in patients with uncontrolled severe hypertension where the SIT is contraindicated, following discussion with a consultant endocrinologist.

Patients should already have been screened with a random Aldosterone:Renin Ratio (ARR) > 1000

Contraindications
Patients in cardiac or renal failure particularly patients on diuretics.

Requirements
- 2 pink top EDTA for plasma renin and aldosterone
- 25-50 mg captopril to be given orally

PATIENT PREPARATION

- Stop mineralocorticoid receptor antagonists (spironolactone and eplerenone) for 6 weeks before the test
- Stop diuretics 4 weeks before the test.
- Stop beta blockers, calcium channel antagonists, ACE inhibitors and AT2 blockers for 2 weeks before the test.
- Can continue to use alpha blockers to manage hypertension. Alternative antihypertensives that can be taken are doxazosin, slow release verapamil, hydralazine with slow release verapamil (to avoid reflex tachycardia)
- Ensure plasma K in normal range (ideally >4) prior to performing test
- Examine patient for signs of cardiac failure. Check BP.
- Patient should be seated for 1 hr before test

Procedure
- Patient should be seated throughout test.
- Perform blood pressure monitoring at 0, 60 and 120mins during test

Minutes	Procedure	Sample
0	Take sample for **Aldosterone and renin** 25-50mg captopril to be given orally	1 x pink top EDTA tube (Aldosterone and renin)
120	Take sample for **Aldosterone and renin**	1 x pink top EDTA tube (Aldosterone and renin)

Interpretation of results[1]

Suppression of aldosterone >30% at 120mins from baseline normal response to captopril

In patients with hyperaldosteronism, aldosterone remains elevated and plasma renin suppressed.

Differences in response can be seen in APA (aldosterone producing adenoma) compared to IHA (idiopathic adrenal hyperplasia); in IHA some decrease in aldosterone may be seen as adrenal hyperplasia is angiotensin II responsive.

Note a substantial number of false positives and equivocal results have been reported with this test.

Reference[1]: Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline. JCEM 2016

Overnight Dexamethasone Suppression Test (ONDST)

In normal subjects, dexamethasone suppresses ACTH and therefore cortisol secretion. In Cushing's syndrome, there is incomplete suppression.

Indications

Screening test for subjects suspected of Cushing's syndrome.

Contraindications

- Patients on enzyme inducing drugs e.g. anti-convulsants may rapidly metabolise dexamethasone.
- Oestrogens (e.g. pregnancy, HRT or COC) may induce cortisol binding protein and artefactually increase total cortisol levels.
- **Urine collection for 24 hr urinary free cortisol must not occur during or on the day following this test.**

Side Effects

None

Requirements

- 1 mg dexamethasone tablet
- 1 brown top serum tube

Procedure

The patient to take 1 mg dexamethasone orally at 23:00 and the following morning at 09:00 a blood sample to be taken for serum cortisol (brown top tube).

Interpretation of results

A normal response is shown by suppression of 09:00 cortisol to <50 nmol/L.

Failure to suppress is seen in the autonomous secretion of cortisol found in Cushing's syndrome. With this cut off, there will be a high false positive rate.

For patients who fail to suppress to <50 nmol/L the laboratory will analyse the sample for dexamethasone:

Dexamethasone <3.0 nmol/L suggests impaired absorption or excess metabolism of dexamethasone, an alternative biochemical screening test to investigate hypercorticolism should be considered. May indicate dexamethasone has not been taken if non compliance is suspected.

Dexamethasone ≥3.0 nmol/L is consistent with adequate absorption and metabolism of dexamethasone.

Sensitivity and specificity

Using a cortisol of 50 nmol/L as the test cut-off, provides sensitivity of 95% with 80% specificity (Wood et al. (1997) Ann Clin Biochem 34:222-229). Specificity is increased to 95% if the cut-off is raised to 140nmol/L (Pecori et al/ (2007) Clin Endocrinol 66:251-257).

If there is strong clinical or biochemical evidence for Cushing's syndrome, a 48h low dose dexamethasone test should be performed as this is more specific.

Normal subjects rarely (2%) fail to suppress with overnight dexamethasone unless they are depressed (10-50%), obese (10%) or systemically unwell (10-20%).

Low Dose Dexamethasone Suppression Test (LDDST)

In normal subjects, dexamethasone suppresses ACTH and therefore cortisol secretion. Patients with Cushing's syndrome, from whatever cause, lose the normal negative feedback control by circulating glucocorticoids on ACTH release and thus exhibit detectable plasma ACTH and cortisol concentrations after dexamethasone administration.

Indications

Screening test for Cushing's syndrome, especially if the result of the overnight suppression test is inconclusive.
In women with a high testosterone this test may be used to differentiate PCOS and partial hydroxylase deficiencies (CAH) from autonomous androgen secreting tumours.

Contraindications

- Patients on enzyme inducing drugs e.g. anti-convulsants may rapidly metabolise dexamethasone.
- Oestrogens (e.g. pregnancy, HRT or COC) may induce cortisol binding protein and artificially increase total cortisol levels.
- Care in diabetes mellitus and patients who are psychologically unstable.

Side Effects

None

Requirements

- A total of eight doses of dexamethasone should be written up (0900, 1500, 2100, 0300, 0900, 1500, 2100, 0300 and **must adhere strictly to the 6-hourly dosing frequency, especially important not to omit or delay the 0300 dose**) Adult dose 0.5mg
- 2 x brown top serum tubes for cortisol
- 2 x orange top lithium heparin tubes for ACTH which must be sent to the laboratory immediately, preferably on ice. If ice not available then sample must be received in laboratory within 1 hr of collection.

PATIENT PREPARATION

Stop all oral oestrogen therapy 6 weeks prior to test. Patients on sex steroid implants might generate results that are difficult to interpret. Measuring SHBG might be helpful in this circumstance.

Procedure

Time	Procedure	Samples
Day 1		
0845	Take basal samples for **cortisol** and **ACTH**	1 x brown top serum tube (cortisol) 1 x orange top Li Hep plasma (ACTH) – send immediately
0900	Patient takes 0.5mg dexamethasone p.o	
1500	Patient takes 0.5mg dexamethasone p.o	
2100	Patient takes 0.5mg dexamethasone p.o	
Day 2		
0300	Patient takes 0.5mg dexamethasone p.o	
0900	Patient takes 0.5mg dexamethasone p.o	
1500	Patient takes 0.5mg dexamethasone p.o	
2100	Patient takes 0.5mg dexamethasone p.o	
Day 3		
0300	Patient takes 0.5mg dexamethasone p.o	
0900	Take samples for **cortisol** and **ACTH**	1 x brown top serum tube (cortisol) 1 x orange top Li Hep plasma (ACTH) – send immediately

Interpretation of results
If the 0900h cortisol value on day 3 (T=48hrs) is <50nmol/L the patient has shown appropriate suppression.

Failure to suppress is seen in the autonomous secretion of cortisol found in Cushing's syndrome. However, since there are several common conditions associated with impaired cortisol suppression following a LDDST (e.g. morbid obesity, depression), the result should always be interpreted in conjunction with the degree of clinical suspicion.

In patients who fail to suppress, a pre-test ACTH level of <5ng/L is highly suggestive of an adrenal cause of Cushing's syndrome.

In virilisation from PCOS or partial hydroxylation deficiencies there will be complete/partial suppression of testosterone. This is not seen in ovarian or adrenal tumours.

Sensitivity and Specificity
Suppression in patients with Cushing's syndrome is rare (2-5%). Sensitivity for Cushing's Syndrome is above 95% with a reported specificity of 70%. Some reported cases metabolise dexamethasone slowly and so achieve higher circulating levels than expected. This test is more specific than the overnight suppression test with a lower false positive rate. Failure of suppression in patients may be seen in patients with systemic illness, endogenous depression, or on enzyme inducing drugs e.g. phenytoin or rifampicin. The predictive value of all tests is falling as morbid obesity becomes more common.

High Dose Dexamethasone Suppression Test (HDDST)

Indications

Patients with definite Cushing's syndrome established by screening but aetiology (Cushing's disease, ectopic ACTH or adrenal adenoma/carcinoma) needs to be further differentiated.

The pre-test probability of ACTH-dependent Cushing's syndrome being secondary to pituitary-dependent Cushing's disease is 85-90%. The HDDST correctly identifies 69% of patients as having Cushing's disease. Since the diagnostic accuracy of this test in identifying Cushing's disease is less than the pre-test probability of making this diagnosis; this test is now rarely used in practice. If ACTH-dependent Cushing's syndrome has been diagnosed following a LDDST, IPSS is used to confirm pituitary localisation and exclude an ectopic source of ACTH.

Contraindications

- Patients on enzyme inducing drugs e.g. anti-convulsants may rapidly metabolise dexamethasone.
- Oestrogens (e.g. pregnancy, HRT or COC) may induce cortisol binding protein and artefactually increase total cortisol levels.
- Take care in patients with severe depression or hypomania.

Requirements

- A total of eight doses of dexamethasone should be written up (0900, 1500, 2100, 0300, 0900, 1500, 2100, 0300) and **must adhere strictly to the 6-hourly dosing frequency.** Adult dose 2mg
- 2 x brown top serum tubes for cortisol
- 2 x orange top lithium heparin tubes for ACTH

PATIENT PREPARATION

- Stop all oral oestrogen therapy 6 weeks prior to test. Implants can cause problems.

Procedure

Time	Procedure	Samples
0845	Take basal samples for **cortisol** and **ACTH**	1 x brown top serum tube (cortisol) 1 x orange top Li Hep plasma (ACTH) – send immediately
0900	Patient takes 2mg dexamethasone	
1500	Patient takes 2mg dexamethasone	
2100	Patient takes 2mg dexamethasone	
0300	Patient takes 2mg dexamethasone	
0900	Patient takes 2mg dexamethasone	
1500	Patient takes 2mg dexamethasone	
2100	Patient takes 2mg dexamethasone	
0300	Patient takes 2mg dexamethasone	
0900	Take samples for **cortisol** and **ACTH**	1 x brown top serum tube (cortisol) 1 x orange top Li Hep plasma (ACTH) – send immediately

Interpretation of results

If the 0900h cortisol is less than 50% of the basal value after 48 hours of dexamethasone this is classified as showing suppression.
Suppression with high dose dexamethasone is usually seen in Cushing's disease but not in ectopic ACTH production or adrenal tumours.

The high dose dexamethasone test is useful but not totally reliable in the differential diagnosis of Cushing's syndrome as it is neither very sensitive nor specific. Suppression occurs in 75% of patients with Cushing's disease, 10-25% of patients with ectopic ACTH and 0-6% of patients with adrenal tumours. Patients with ectopic ACTH who show suppression tend to have occult and relatively benign tumours with lower levels of ACTH and cortisol. These patients are very hard to differentiate from Cushing's disease.

The 0900h cortisol after 48 hours is considered to be the best parameter to use to discriminate between Cushing's disease and ectopic ACTH. The criterion of 50% suppression at 48 hours should not be applied too rigidly as many cases of Cushing's disease will suppress by 40 or 45% or suppress after 72 hours. In difficult cases it is advisable to repeat the test as no patients with an adrenal tumour have been shown to have reproducible suppression and cases of Cushing's syndrome may show cyclical variation.

Cortisol Day Curve on Hydrocortisone

Indications
Establish correct dose and distribution through the day of the replacement dose of hydrocortisone (n.b. this has no value in patients taking prednisolone). Some hepatic enzyme inducers such as Rifampicin, Phenobarbitone and Phenytoin will increase clearance of hydrocortisone and may lead to problems with maintenance therapy.

Contra-indications
None

Requirements
- IV cannula
- Patient's hydrocortisone therapy
- 5 x brown top serum tubes

Procedure

PATIENT PREPARATION

- Oral oestrogen therapy must be stopped 6 weeks before the day curve otherwise it is difficult to interpret because of oestrogen induced rise in CBG.
- **It is essential to clearly explain and remind the patient not to take their morning dose of hydrocortisone until the first blood sample is taken**
- Timing of samples will vary depending on dose regime, example timings given below
- Record time of hydrocortisone dose and time each sample is collected

Sample No.	Approx Time	Procedure	Samples
1	On patient Arrival	Insert IV cannula Take sample for **cortisol pre-dose**	1 x brown top serum tube
		Patient should take normal morning dose of hydrocortisone	
2	0900	Take sample for **cortisol**	1 x brown top serum tube
3	1230	Take sample for **cortisol**	1 x brown top serum tube
		Patient should take afternoon dose of hydrocortisone	Please note, this time may vary between patients
4	1400	Take sample for cortisol	1 x brown top serum tube
		Patient should take afternoon dose of hydrocortisone	Please note, this time may vary between patients
5	1730	Take sample for **cortisol**	1 x brown top serum tube

Note: if additional samples are required then these can be sent to the laboratory as further individual cortisol requests in EPIC (only 5 samples included in DFT).

Interpretation of results

Aim for adequate cortisol levels throughout the day (peak <900 nmol/L, trough >100 nmol/L).

As a guide, the values below are commonly found. Minor departures do not necessarily need dose adjustment, especially if the patient is well:

- Morning peak cortisol 500 – 800 nmol/L
- Lunchtime peak cortisol 400 – 500 nmol/L
- Post evening dose 300 – 400 nmol/L

Day Curve Chart

DAY CURVE TYPE – please state e.g. Hydrocortisone, Metyrapone, Growth Hormone	

Please fill in below the time that any samples are taken and what they are for and the time, dose, and type of any medication taken by the patient.

ATTENTION:
A pre-dose sample is always required

Sample number	Time sample taken	Type of medication and dose	Time medication taken
1 (Pre-dose)	:		:
2	:		:
3	:		:
4	:		:
5	:		:

Note: if additional samples are required then these can be sent to the laboratory as further individual cortisol requests in EPIC (only 5 samples included in DFT). Include details below.

Additional samples: YES/NO Time sample taken:

Cortisol Day Curve on Metyrapone

Indications
Assessment of biochemical control of Cushing's disease on Metyrapone.
To provide an indication of the average concentration of cortisol to which the tissues are exposed.

Contraindications
None required.

Requirements
- IV cannula
- Patient's metyrapone therapy
- 5 x brown top serum tubes

Procedure

PATIENT PREPARATION

- Patients are NOT required to fast prior to testing
- Oral oestrogen therapy must be stopped 6 weeks prior to the day curve otherwise it is difficult to interpret because of the oestrogen induced rise in CBG
- Timing of samples may vary for each patient, example timings given below
- Record time and dose of medications and time each sample is collected.

Sample No.	Time	Procedure	Sample
	On arrival	Insert *iv* cannula	
1	09:00	Take sample for **cortisol**	1 x brown top serum tube
2	12:00	Take sample for **cortisol**	1 x brown top serum tube
3	15:00	Take sample for **cortisol**	1 x brown top serum tube
4	18:00	Take sample for **cortisol**	1 x brown top serum tube
5	21:00	Take sample for **cortisol**	1 x brown top serum tube

Interpretation of results

A mean serum cortisol between 150 and 300 nmol/L is compatible with a normal production rate. Patients with a higher mean value generally require an increase in therapy, and patients with a lower mean value a reduction.

11 Deoxycortisol and other cortisol precursors accumulate in patients on metyrapone and they cross-react in the cortisol assays, therefore samples are requested for measurement of cortisol by mass spectrometry in these patients.

Growth Hormone Suppression Test

GH secretion is part of the counter-regulatory defence against hypoglycaemia and physiological GH secretion is inhibited by hyperglycaemia. In acromegaly, or gigantism, GH secretion is autonomous and does not suppress and may paradoxically rise with hyperglycaemia.

Indications
This is the gold standard investigation to establish the biochemical diagnosis of acromegaly or gigantism. This test is also used to assess response to medical/surgical treatment of acromegaly.

Side Effects
Some subjects feel nauseated and may have vaso-vagal symptoms during this test.

Requirements

- Adults POLYCAL® 113mL or 75g anhydrous glucose (made up to 200mL with water) plus 100mL cold water, total 300mL
- 6 x yellow top fluoride EDTA tubes, 7 x brown top serum tubes
- Indwelling cannula.

Procedure

PATIENT PREPARATION

The patient should from midnight (sips of water allowed) and should rest throughout the test.

Minutes	Procedure	Sample
0	Insert cannula and take samples for **growth hormone, glucose and IGF-1** Drink glucose solution/polycal within 5 minutes	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH) 1 x brown top serum (IGF-1)
30	Take samples for **Growth Hormone and Glucose**	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH)
60	Take samples for **Growth Hormone and Glucose**	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH)
90	Take samples for **Growth Hormone and Glucose**	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH)
120	Take samples for **Growth Hormone and Glucose**	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH)
150	Take samples for **Growth Hormone and Glucose**	1 x yellow top fluoride EDTA (glucose) 1 x brown top serum (GH)

Ensure the patient is given food and drinks before discharge.

Interpretation of results

Normal subjects will exhibit suppression of GH to <0.3 µg/L[2]

Failure of suppression or a paradoxical rise in GH suggests acromegaly.

NB paradoxical rise in GH may occur during GTT during normal adolescence.

Sensitivity and specificity

GH may fail to suppress due to chronic renal failure, liver failure, active hepatitis, anorexia nervosa or other causes of chronic starvation, malnutrition, hyperthyroidism, diabetes mellitus and in adolescence.

Reference[2]: Automated 22-kD Growth Hormone-specific assay without interference from Pegvisomant. Manolopoulou J et al. Clin Chem 58:10; 1446-1456 (2012)

Growth Hormone Day Curve

Indications

To assess clinical severity of growth hormone excess in acromegaly and to assess response to medical or surgical treatment.

Contraindications
None

Side Effects
None

Requirements
- Indwelling cannula
- 4 x brown top serum tubes

Procedure
- The number of samples and timing may vary for each patient, examples given below.
- Record time each sample is collected.

Sample No.	Time	Procedure	Sample
	08:45	Insert cannula and allow the patient to rest for 15 mins so that stress does not interfere with the results	
1	09:00	Take sample for Growth Hormone and IGF-1	1 x brown top serum tube (GH) 1 x brown top serum tube (IGF-1)
2	12:00	Take sample for Growth Hormone	1 x brown top serum
3	15:00	Take sample for Growth Hormone	1 x brown top serum
4	18:00	Take sample for Growth Hormone	1 x brown top serum
5	Additional time point if required	Take sample for Growth Hormone	1 x brown top serum

Interpretation of results

The mean Growth Hormone level should be <1.7 µg/L

Well controlled patients in remission should have at least one suppressed level <0.3 µg/L

Arginine Stimulation Test

Arginine stimulates growth hormone secretion in healthy individuals but response will be impaired in hypopituitarism.

Indications

Investigation of growth hormone reserve in children and as a secondary confirmatory test in adults or in cases where the insulin tolerance test and glucagon test are contra-indicated.

Side effects and Precautions

- Some adolescents may need sex hormone priming before this test. Please check with the requesting doctor.
- Arginine can cause nausea and some irritation at the infusion site and the patient should be made aware of this.
- Arginine can cause vasospasm so sampling may be difficult if only one cannula is used. For this reason large veins should be selected.
- Ensure patient is recumbent during procedure (BP may fall by 20-30 mmHg in first 30 mins).

Requirements

- Arginine 2 x 100ml of 20% (each 100ml bottle contains 20g L-Arginine) (0.5g/kg – max dose 40 g)
- Normal saline (0.9% saline)
- 5 brown top serum tubes
- *iv* cannula

Procedure

PATIENT PREPARATION

- If the patient is on growth hormone replacement, this should be stopped for one month before testing.
- Fast the patient from midnight before the test (water is allowed).
- Weigh the patient, document accurately in the medical notes and calculate dose.

Minutes	Procedure	Samples
-30	Insert an indwelling cannula into each arm (one for infusion and one to take blood from) Allow the patient to rest for at least 30 mins.	
0	Take basal sample for **growth hormone**.	1 x brown top serum tube
0	Infuse L-arginine hydrochloride over 30 minutes at a dose of 0.5g/kg (max 40g) in 500mLs 0.9% saline	
30	Take sample for **growth hormone**	1 x brown top serum tube
60	Take sample for **growth hormone**	1 x brown top serum tube
90	Take sample for **growth hormone**	1 x brown top serum tube
120	Take sample for **growth hormone**	1 x brown top serum tube

Interpretation of results

Adults: GH should rise to at least 5.3 µg/L
GH levels of <3 µg/L suggest severe growth hormone deficiency

Cannulated prolactin

Indication

To exclude stress and/or fear of venepuncture as cause of previously identified hyperprolactinaemia.

Contraindications
None

Side Effects
None

Requirements
- Indwelling cannula
- 3 x brown top serum tubes

Procedure

PATIENT PREPARATION

Patient should rest throughout the test to ensure that stress does not interfere with the test.

Minutes	Procedure	Sample
0	Insert cannula and immediately take sample for Prolactin, LH, FSH, Oestradiol (if Female) or Testosterone (if male)	1 x brown top serum tube
30	Take sample for Prolactin	1 x brown top serum
90	Take sample for Prolactin	1 x brown top serum

Interpretation of results

Prolactin should decrease from an initial high concentration to normal during the test.
A lack of decrease in prolactin over 90 mins excludes stress as cause of the hyperprolactinaemia and will require further investigation.

Water Deprivation Test

This is potentially dangerous and must be undertaken with great care. Patients unable to conserve water may become critically dehydrated within a few hours of water restriction.

Water restriction in the normal individual results in secretion of arginine vasopressin (AVP) from the posterior pituitary in order to reabsorb water from the distal renal tubules and concentrate the urine. Failure of this mechanism results in a rise in plasma osmolality due to water loss, and a dilute urine of low osmolality.

The two causes are a. A failure of AVP secretion (central DI)
b. Insensitivity of the renal tubules to AVP (nephrogenic DI)
The cause may be distinguished by the administration of DDAVP (synthetic AVP).

Indications

Investigation of suspected central diabetes insipidus (CDI), nephrogenic diabetes insipidus (NDI) and primary polydipsia (PP).

Contraindications
If there is evidence of the kidney's ability to concentrate the urine e.g. spot urine osmolality >750mOsm/kg.

Exclude other causes of polyuria (e.g. diuretics, chronic kidney diseae, hypercalcaemia, hypokalaemia, diabetes mellitus, UTI, therapy with carbamazepine, chlorpropamine, lithium). Anterior pituitary hormone deficiency renders results meaningless as, in particular, steroid and thyroxine deficiencies impair excretion of a free water load.

Precautions and preparations
Patients should not have any access to any food or drink throughout the test and must be closely monitored throughout the test to ensure this.
Inform the Duty Biochemist at least 1 day in advance of performing this procedure so that samples can be processed efficiently.

Side Effects
Patients with true diabetes insipidus may become severely water depleted during water deprivation and MUST be carefully monitored (by weighing and quantifying urine output regularly) throughout the test.
Patients suspected of having primary polydipsia may become severely hyponatraemic if they drink excessively after being given DDAVP, so MUST NOT drink more than 500mls fluid in total over the following 8 hours.

Requirements
- Accurate scales for weighing the patient
- Sarstedt urine containers for urine osmolality
- Brown top serum tubes for serum osmolality
- Urine measuring jug
- DDAVP 2µg IM

Procedure

PATIENT PREPARATION:

Patient should be admitted on the day before the test.

1. If the patient is on DDAVP, this is discontinued the evening before the test.
2. Monitor the patient's fluid balance for a complete 24 hour period the day before the test to accurately quantify fluid intake and output.
3. If indicated give normal steroid and/or thyroid hormone replacement before the test.
4. Tea, coffee, alcohol and tobacco are specifically excluded after midnight before the test and during the test because they directly stimulate (vagus) the secretion of AVP independently of the osmoreceptors.
5. Patient is allowed to drink freely until the start of the test i.e. 08.00h.
6. A light breakfast is permitted before test commences e.g. 07.00h.

TEST

1. Complete the Water Deprivation test template during the test.
2. At 08.00h the patient should empty their bladder and this urine should be discarded
3. 09.00h commence fluid restriction, weigh the patient and calculate 97% of their weight. Begin the fluid balance chart. Take urine and serum samples for osmolality and serum for sodium (Na). Urine volume should be measured throughout the test.
4. 12.00h, 14.00h, 15.00h the patient should be weighed and samples taken for serum and urine osmolality and serum Na and sent directly to the lab labelled correctly and including clinical details so that the tests can be prioritised.

INDICATIONS FOR STOPPING THE TEST:
- **Weight loss is >3% of initial weight**
- **Serum osmolality rises to >300 mOsmol/kg**

5. Review the results. If urine osmolality is <750 mOsm/kg or if urine osmolality failed to rise by more than 30 mOsm/kg over 3 successive urine samples, then administer 2µg DDAVP IM at 16.00h and allow food and fluids.
6. Check urine osmolality at 2hr and 4hr post-DDAVP and the next morning.

AFTERCARE

- Keep the patient in overnight for observation and issue the patient information leaflet for Water Deprivation Tests.

WARNING: After completing the water deprivation test patients should NOT consume **>500ml fluid** for **8 hours**

Interpretation of results

Post-Dehydration Osmolality (mOsm/kg)		Post DDAVP osmolality (mOsm/kg)	Diagnosis
Serum	**Urine**	**Urine**	
<300	>750	>750	Normal
>300	<300	<300	Nephrogenic DI
>300	<300	>750	Central DI
<300	300-750	<750	Primary Polydipsia or partial CDI

NB. chronic primary polydipsia can dissipate the renal medullary osmotic gradient, thereby reducing the renal response to endogenous and exogenous AVP.

In severe central DI, maximal urinary concentration may be achieved only after repeated DDAVP.

EQUIVOCAL RESULTS

Many patients fall in the range 300-750 following water deprivation and it is often difficult to differentiate between PP and partial DI, especially following pituitary-surgery. In this instance, the plasma sodium may be helpful, since in PP, this is often low at the start of the test.

If there is a partial response, this test does not reliably differentiate between PP and partial CDI, and may indicate that the patient has been drinking during the test. In these cases the test can be repeated fasting the patient from midnight the night before the test.

If results are equivocal and there remains clinical suspicion of DI then proceed to hypertonic saline infusion test or Arginine Stimulated Co-peptin test.

Elderly patients may not achieve maximal concentration of their urine and therefore results should be interpreted on a case by case basis.

SENSITIVITY AND SPECIFICITY

When correctly performed, the water deprivation test has a sensitivity and specificity of 95% for diagnosing and differentiating severe central DI and nephrogenic DI. The incidence of false positive and false negative results for PP or partial CDI/NDI is 30-40% (investigate further).

WATER DEPRIVATION TEST TEMPLATE

Patient Name:		**Sex:**	
Hospital No:		**DOB:**	
Today's Date:			

Perform test under strict supervision to ensure the patient has no access to fluids

The test must be stopped if weight loss exceeds 3%, or if serum osmolality rises above 300 mOsm/kg

Weight patient: **kg** **Calculate weight minus 3%:** **kg**

Time							
0 Hrs	:	**Take sample for urine and serum osmolality/Na and send to the lab.**					
		Weight kg Loss >3%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes/No	**Serum Na mmol/L**	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+3 Hrs	:	**Take sample for urine and serum osmolality/Na and send to the lab.**					
		Weight kg Loss >3% Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes/No	**Serum Na mmol/L**	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+5 Hrs	:	**Take sample for urine and serum osmolality/Na and send to the lab.**					
		Weight kg Loss>3%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes/No	**Serum Na mmol/L**	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+6 Hrs	:	**Take sample for urine and serum osmolality/Na and send to the lab.**					
		Weight kg Loss >3%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes/No	**Serum Na mmol/L**	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**

If the urine osmolality is >750 mOSM/kg STOP the test
If urine osmolality is <750mOSM/kg or has failed to rise by >30mOSM/kg over 3 successive urines then **administer 2µg DDAVP IM** and fill in the table below.

DDAVP	:	**Record time that DDAVP was Administered**	Allow food and minimal fluids **DO NOT** allow excessive drinking
+2hrs	:	Collect urine for osmolality and send to the lab Result: mOsm/kg	
+4hrs	:	Collect urine for osmolality and send to the lab Result: mOsm/kg	

Hypertonic Saline Infusion Test for Diabetes Insipidus

This is potentially dangerous and must be undertaken with great care. Patients unable to conserve water may rapidly become severely hypertonic during this test

An increase in serum osmolality is a strong stimulus for AVP release via the hypothalamic osmoreceptors. Administration of hypertonic saline intravenously will produce a hyperosmolar state, causing maximal stimulation of AVP secretion. Serum copeptin, the C-terminal glycoprotein of the AVP prohormone, is measured as a more stable marker of AVP secretion and is present in approximately equimolar concentrations. Serum copeptin is reported in relation to serum osmolality and assessed using a normogram. Serum copeptin and AVP levels have been shown to agree well.

Indications

To make a clear diagnosis of central diabetes insipidus (CDI) in subjects with polyuria and normal serum osmolality.
This is a specialist investigation and should only be conducted after referral to Endocrinology and performed as an inpatient.
Nephrogenic DI may be investigated using a paired random co-peptin and osmolality with co-peptin levels > 21.4pmol/L suggestive of nephrogenic DI.

Contraindications

Patients with epilepsy, cerebral or cardiovascular disease.

Side Effects

There is a serious risk of dehydration in patients with DI.
The hypertonic saline may induce thrombophlebitis at the site of the infusion.

Requirements

- 14 x brown top serum sample tubes
- 5% saline
- *iv* cannula
- (note orange Li Hep tubes also acceptable for co-peptin

Procedure

PATIENT PREPARATION

Fast from midnight prior to the test. Water only to be consumed, no more than 500ml. No tea, coffee, alcohol or smoking after midnight.

Weigh the patient.

TEST

Inform the Biochemistry laboratory that the samples for copeptin will be arriving so that samples can be processed promptly. Samples should arrive within 2hrs.

- A thirst chart should be completed throughout the test
- BP should be measured at regular intervals throughout the test

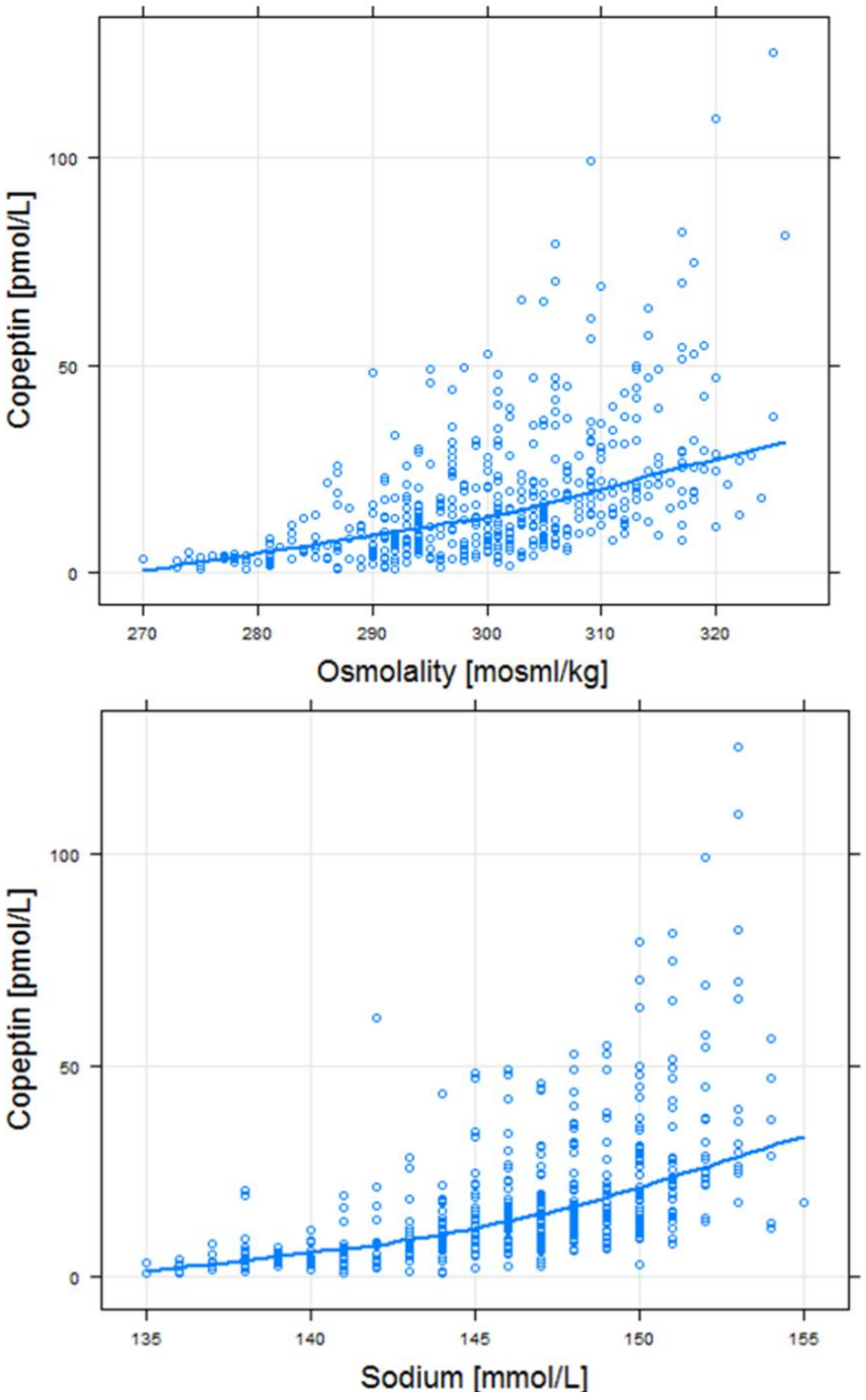
Minutes	Procedure	Samples
-30	Basal Sample	1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
0	Infuse 5% saline 0.06ml/kg/min for 2 hours	1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
30		1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
60		1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
90		1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
120	STOP SALINE INFUSION	1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
135		1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)

Note Copeptin samples are referred to Royal Victoria Hospital, Newcastle-upon-Tyne, for analysis and reported versus a normogram.

Interpretation of results
Patients with primary polydipsia have normal copeptin rise in response to the hyperosmolar state induced by the procedure. Patients with central diabetes insipidus have little or no rise in copeptin.

Results that fall within the central area of the graph indicate a normal response. Points falling to the right show decreased copeptin (and therefore AVP) relative to normal osmoregulation and indicate central DI.

Correlation of Co-peptin levels with plasma osmolality and sodium results from 91 healthy individuals (blue data points)[3]



Reference[3]

Schnyder et al.(2015) Physiological area of normality of co-peptin in normal-to-hyperosmolar states. Endocrine Abstracts 37:EP706.

Arginine-stimulated Co-peptin test for Diabetes Insipidus

Administration of arginine acts as a stimulus to the posterior pituitary thus increasing AVP secretion. Serum copeptin, the C-terminal glycoprotein of the AVP prohormone, is measured as a more stable marker of AVP secretion and is present in approximately equimolar concentrations. Serum copeptin and AVP levels have been shown to agree well.

Indications

The primary goal of this test is to diagnose Diabetes Insipidus (DI) and specially to distinguish the different entities of Hypotonic Polydipsia- polyuria Syndromes (Central / Nephrogenic DI and Primary Polydipsia) particularly if hypertonic saline test is contra indicated.

Contra indications

If diagnosis and treatment of DI is urgent consider Water deprivation test or hypertonic saline infusion test, as the usual turnaround time for co-peptin results is 3 weeks.

Random co-peptin levels > 21.4pmol/L suggests Nephrogenic DI and if levels < 21.4pmol/L to proceed with Arginine stimulated co-peptin test.

Side effects

- Arginine can cause nausea and some irritation at the infusion site and the patient should be made aware of this.
- Potential rare side effects include headache, vomiting, vertigo and facial paraesthesia and to be noted in case record.
- Arginine can cause vasospasm so sampling may be difficult if only one cannula is used. For this reason large veins should be selected.
- Ensure patient is recumbent during procedure (BP may fall by 20-30 mmHg in first 30 mins).

Requirements

- Arginine 2 x 100ml of 20% (each 100ml bottle contains 20g L-Arginine)
- Normal saline (0.9% saline)
- 4 brown top serum tubes
- (note orange Li Hep tubes also acceptable for copeptin)
- *iv* cannula

Procedure

PATIENT PREPARATION

- Stop DDAVP 24 hours beforehand in patients already on treatment. The requesting Endocrine team to supervise the discontinuation and fluid intake advice.
- Ensure patient has fasted from midnight but are allowed to drink fluids until 2 hrs before start of the test.
- If the patient is on any other hormone replacement tablets, these should be taken as usual.

TEST

Inform the Biochemistry laboratory that the samples for copeptin will be arriving so that samples can be processed promptly. Samples should arrive within 2hrs.

- Record Weight, Blood pressure and Pulse at the start of test.
- Insert intravenous cannula in antecubital fossa and allow patient to rest for 30 mins. **Important to insert a PINK/GREEN cannula as Arginine constricts the blood vessels**
- Take baseline blood samples for serum sodium, Osmolality and Co-peptin
- Infuse Arginine over 30 mins, at a dose of 0.5 g/kg bodyweight (max 40g) diluted in 500mls 0.9% saline. Once infused, flush the cannula well with normal saline.
- Take blood samples at 60 mins for serum sodium, Osmolality and Co-peptin.
- Remove cannula.
- Patient can have food, drink and DDAVP (if already taking) after the test is concluded.

Minutes	Procedure	Samples
0 (Baseline)	Take samples for **sodium, osmolality and co-peptin**	1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)
0	Infuse L-arginine hydrochloride over 30 minutes at a dose of 0.5g/kg (max 40g) in 500mLs 0.9% saline	
240	Take samples for **sodium, osmolality and co-peptin**	1 x brown top serum (osmolality, Na) 1 x brown top serum (copeptin)

Note Samples will need to be requested individually in EPIC as not included currently as a requestable DFT. Please record 0 min (Baseline) and 240 min sample in reason for request.

Interpretation

If, at 60 min after Arginine infusion, serum co-peptin is <3.8 pmol/L, then the diagnosis is Central DI

If the serum co-peptin is >3.8 pmol/L, then the diagnosis is likely to be Primary Polydipsia.

Reference

Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study. The Lancet (2019) online http://dx.doi.org/10.1016/S0140-6736(19)31255-3

Oral Glucose Tolerance Test

In normal individuals pancreatic insulin secretion maintains blood glucose within a tight concentration range following an oral glucose load. Failure of insulin secretion, or resistance to insulin action, will result in an elevation in blood glucose.

Indications

Investigation of impaired glucose tolerance or diabetes mellitus.

The diagnosis of diabetes is made on the basis of repeatedly elevated fasting plasma glucose. The use of the oral glucose tolerance test is to clarify borderline elevations in fasting plasma glucose and for those conditions where diagnosis using HbA1c is contraindicated.

Contra-indications

- Patients who are under physical stress e.g. post surgery, trauma or infection or extreme psychological stress as these may give misleading results.
- Patients with hypokalaemic periodic paralysis.

Side Effects

Some subjects feel nauseated and may have vasovagal symptoms during this test.

Requirements

- POLYCAL 113mL or 75g anhydrous glucose made up to 200 mL with water plus 100mL cold water, total 300mL
- 2 x yellow top fluoride EDTA tubes

Procedure

PATIENT PREPARATION

- Patients should be advised to eat a normal carbohydrate diet (>150g daily) for at least 3 days prior to the test and undertake normal physical activity.
- Patients must fast from midnight prior to this test but may drink small volumes of plain water.
- Smoking and physical exercise should NOT be allowed in the morning prior to, and during, the test.

This test should be performed in the morning. Patients should remain at rest during the test.

TEST

Minutes	Procedure	Samples
0	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
0	Polycal should be drunk over 5 minutes followed by the water	
120	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)

Interpretation of results

	Plasma Glucose (mmol/L)		
	0 minute		**120 minute**
Non Diabetic	<6.1	and	<7.8
Impaired fasting glucose	6.1 - 6.9	and	<7.8
Impaired glucose tolerance	<7.0	and	7.8 - 11.0
Diabetes mellitus	7.0 or greater	and/or	11.1 or greater
In pregnancy according to MFT GDM (HAPO) guidelines 2021:			
Consistent with Gestational diabetes mellitus	5.3 or greater	and/or	8.5 or greater

72 hour Fast for Insulinoma Investigations

Prolonged fasting is a sensitive procedure for detection of endogenous hyperinsulinism (sensitivity >90 %) and is routinely employed as the initial test to detect inappropriately elevated insulin secretion as the cause for recurrent hypoglycaemia.

Indications

Used to demonstrate fasting hypoglycaemia and diagnosis of insulinoma if not observed spontaneously or after an overnight fast.

Requirements

- Yellow top fluoride EDTA sample tubes for glucose
- Brown top serum sample tubes for insulin and C-peptide
- Iced slurry for transportation of samples.
- Indwelling cannula
- Perform test under close supervision
- 10% dextrose (250ml) or glucagon 1mg available for immediate administration for hypoglycaemia.
- Orange juice or Gluco juice available for treatment of hypoglycaemia

Procedure

PATIENT PREPARATION

- The onset of the fast is classed as the last intake of calories.
- Calorie free, caffeine free beverages only may be consumed.
- Prescribed medication can be continued**.**
- **Smoking is not permitted during the test.**
- The patient should remain physically active during waking hours, but not leave the ward.

TEST

- Cannulate patient and commence prolonged fast.
- Bedside capillary blood glucose monitoring must be performed every 4 hours or when clinical symptoms are reported and signs of hypoglycaemia are observed (sweating, palpitations, anxiety, faintness) to assess the degree of hypoglycaemia.
- Each time blood glucose is checked a blood ketone level should be measured and recorded in the patient notes.
- If the **bedside capillary blood glucose result is found to be <3mmol/L or there are symptoms of hypoglycaemia** then samples must be taken immediately to send to the laboratory for plasma glucose sample (yellow top fluoride EDTA tube) accompanied by insulin and c-peptide samples (2 x brown top serum tubes on ice). **Send to the laboratory immediately after collection as urgent samples.**
- If the laboratory glucose level is found to be <2.2 mmol/L or the patient shows severe symptoms/ signs of hypoglycaemia then carbohydrate should be given or 250ml 10% dextrose IV or 1mg Glucagon IM/IV/SC, see Trust policy on treatment of hypoglycaemia in adults, and the fast should be stopped.

NB Insulin and c-peptide samples will only be analysed when laboratory glucose <2.5mmol/L.

Tests set up in EPIC

Sample 1 (Admission)	Sample type	Samples 2 – 18 *
Glucose	Yellow top fluoride EDTA tube	Glucose
Insulin	Brown top serum tube	Insulin
C-peptide	Brown top serum tube	C-peptide
FFA	Brown top serum tube Send to laboratory on ice, must be received within 20 mins of collection	FFA
B-OHB	Brown top serum tube	B-OHB
Cortisol	Brown top serum tube	
Sulfonylurea screen	Brown top serum tube	
Exogenous Insulin	Brown top serum tube	

***Note** should the patient become hypoglycaemic (glucose <2.5 mmol/L) then additional samples for sulfonylurea screen and exogenous insulin at that time point should be taken and requested in EPIC.

Interpretation of results

ORC In normal individuals plasma glucose should not fall below 2.2mmol/L, with serum insulin and c-peptide levels appropriate for glucose level.
The diagnosis of insulinoma rests on the demonstration of hypoglycaemia by laboratory plasma glucose ≤2.2mmol/L

Glucose ≤2.2 mmol/L:

Insulin <3 pmol/L	No evidence of inappropriate insulin secretion
Insulin 3-12 pmol/L	Inappropriate insulin secretion NOT excluded. Interpret results alongside other biochemical and clinical parameters.
Insulin >12 pmol/L	Results suggestive of insulin secretion

Sensitivity and specificity

By 24 hrs, 66% insulinomas develop hypoglycaemia and by 48 hrs, >95% insulinomas can be diagnosed. After 72 hrs fast plus exercise, if no hypoglycaemia, insulinoma is very unlikely.

Mixed Meal Test for Postprandial Hypoglycaemia

Indications
For use in patients with suspected postprandial hypoglycaemia, or in conjunction with prolonged supervised fast in patients with suspected insulinoma. A proportion of patients with insulinoma will show a positive test (approx. 6%)[4].
Endocrine Society guidelines do not recommend the use of prolonged oral glucose tolerance test for the diagnosis of reactive hypoglycaemia[5,6]

Contra-indications

None

Requirements

- **ENSURE PLUS MILK SHAKE** contains 17% Protein, 29% fat and 50% carbohydrates (CHO) and the standard presentation is 220ml bottle. This will give 330Kcal (13.8g Protein, 10.8g Fat and 44.4g carbohydrates Abbott laboratories, Abbott Park, IL).
- *iv* cannula
- 10 x yellow top fluoride EDTA tubes
- 20 x brown top serum sample tubes for insulin and c-peptide
- 10% dextrose (250ml) or glucagon 1mg available for immediate administration for hypoglycaemia.
- Orange juice or Gluco juice available for treatment of hypoglycaemia

Procedure

PATIENT PREPARATION

- The patient should have been on a diet containing adequate amount of carbohydrate (250g/day) for at least 3 days before the test.
- Patients should be fasted from 10pm prior to the test, water is permitted
- Avoid smoking on the day of test
- If patient is on diazoxide discontinue a week before the test. Other usual medications should be taken.

TEST

- Insert cannula and take blood for baseline plasma glucose at time 0
- Give mixed meal
- Take blood for glucose, insulin and c-peptide at 30, 60, 90, 120, 150, 180, 240, 270 and 300 minutes on ice **Send to the laboratory immediately after each timed collection as urgent samples.**
- Observe patient for symptoms and/or signs of hypoglycaemia. Avoid symptomatic treatment if possible, until the test is completed.
- If the laboratory glucose level is found to be <2.2 mmol/L or the patient shows severe symptoms/ signs of hypoglycaemia then carbohydrate should be given or 250ml 10%

dextrose IV or 1mg Glucagon IM/IV/SC, see Trust policy on treatment of hypoglycaemia in adults, and the fast should be stopped.

Minutes	Procedure	Samples
0	Take sample for **glucose**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
0	**Give mixed meal Ensure Plus**	
30	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
60	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
90	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
120	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
150	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
180	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
240	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
270	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube
300	Take sample for **glucose, insulin and c-peptide**	1 x yellow top fluoride EDTA, 2 x brown top serum tube

Interpretation of results

- A laboratory glucose result <3 mmol/L is consistent with reactive hypoglycaemia and requires follow up.
- **NB** Insulin and c-peptide samples will only be analysed when laboratory glucose <3 mmol/L

There is no consensus on the threshold of hypoglycaemia required for diagnosing reactive hypoglycaemia on a mixed meal test. In practice, the interpretation of the test results is the same as when the tests are done during a spontaneous episode of hypoglycemia or during a 72-hour fast. Therefore inducing symptoms consistent with hypoglycaemia in the presence of a low glucose (usually below 3.0 mmol/L), would constitute a positive test finding.

References

[4]Placzkowski KA, et al 2009 Secular trends in the presentation and management of functioning insulinoma at the Mayo Clinic, 1987-2007. J Clin Endocrinol Metab 94(4): 1069-73

[5]Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, Service FJ 2009 Evaluation and Management of Adult Hypoglycemic Disorders: And Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 94(3): 709-728

[6]Hogan MJ, Service FJ, Sharbrough FW, Gerich JE 1983 Oral glucose tolerance test compared with a mixed meal in the diagnosis of reactive hypoglycemia. A caveat on stimulation. *Mayo Clin Proc* 58:491–496

Levothyroxine Absorption Test

To investigate cause of persistently elevated TSH in primary hypothyroid patients on levothyroxine therapy using oral administration of a high dose of levothyroxine and monitoring TSH and FT4 levels post absorption. Cause may be non-compliance with medication or due to issue with absorption of levothyroxine.

Indication
To investigate patients with persistently elevated TSH despite apparently adequate levothyroxine replacement therapy.

Side effects:
- Possible tachycardia and palpitations. Patient should have observations every 60 mins.

Requirements
- Levothyroxine 1000mcg orally, or total weekly Levothyroxine as a single dose, after discussion with consultant endocrinologist
- iv cannula

Procedure

PATIENT PREPARATION
- Omit usual dose of levothyroxine on day of the test.
- Medication review, document in notes (patient to bring all usual medications).
- 0 hour – cannulate, sample for TSH, FT4
- Give levothyroxine orally as a single dose with water.
- Take bloods for FT4 at 60, 90, 120, 180 minutes.
- Bloods for TSH, FT4 at 240 minutes.

TEST
- Insert cannula and perform observations at baseline and every 60 minutes.

Minutes	Procedure	Samples
0	Insert cannula, perform observations	
0 hours	Take sample for **TSH, FT4**	1 x brown top serum tube
0 hours	Give levothyroxine orally	
60 minutes	Take sample for **FT4** Observations	1 x brown top serum tube
90 minutes	Take sample for **FT4** Observations	1 x brown top serum tube
120 minutes	Take sample for **FT4** Observations	1 x brown top serum tube
180 minutes	Take sample for **FT4** Observations	1 x brown top serum tube
240 minutes	Take sample for **FT4** Observations	1 x brown top serum tube

Note Samples will need to be requested individually in EPIC as not included currently as a requestable DFT. Please record each time in minutest from 0 mins (Baseline) to 240 mins.

Interpretation

Adequate absorption of levothyroxine:

Incremental increase in FT4 at 120mins of ≥54% (+3%)[1] or at 240mins of >60%[2] from baseline

References

[1] A thyroxine absorption test followed by weekly thyroxine administration: a method to assess non-adherence to treatment. Eur J Endocrinol (2013) 168: 913-917

[2]M Utility of the levothyroxine absorption test: the Mayo Clinic experience (2019) 3(Suppl): OR19-3