17 Hydroxyprogesterone Day Curve

Indications:
Assessment of biochemical control in patients with congenital adrenal hyperplasia who are on glucocorticoid treatment.

Contraindications:
None

Requirements

- 6 x brown top serum tubes for 17OHP measurement
- Cannula
- Day Curve form on Page 10

Procedure

PATIENT PREPARATION

- Patients are NOT required to fast prior to testing
- Insert *iv* cannula
- Note time and dose of medications.

TEST

Fill in Day Curve form on page 10

Time	Procedure	Sample
09:00	Take sample for **17OHP**	1 x brown top serum tube
12:00	Take sample for **17OHP**	1 x brown top serum tube
15:00	Take sample for **17OHP**	1 x brown top serum tube
18:00	Take sample for **17OHP**	1 x brown top serum tube
21:00	Take sample for **17OHP**	1 x brown top serum tube
24:00	Take sample for **17OHP**	1 x brown top serum tube

NB: It is also possible for patients to carry out this test at home. In this instance samples from finger pricks are collected onto Guthrie cards at 3 time points throughout the day, the last should be just before bed time. The cards may then be posted back to the Dept.

Interpretation of results
Mean 17OHP should be <30nmol/L

72 Hour Fast – Provocation for Insulinoma

Principle

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

For the diagnosis of Insulinoma.

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.
- 50ml 50% dextrose available for immediate administration for 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

- 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.
- 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, carbohydrate should be given or 50 ml of 50% dextrose should be given iv and the fast should be stopped.
- **NB** Insulin and c-peptide samples will only be analysed when laboratory glucose <2.5mmol/L.

Interpretation of results

Plasma glucose should not fall below 2.2mmol/L, serum insulin and c-peptide levels should be appropriate for glucose level.
The diagnosis of insulinoma rests on the demonstration of hypoglycaemia by laboratory plasma glucose <2.2mmol/L with concurrent serum insulin level >5mU/L.

Arginine Stimulation Test for Growth Hormone

Principle

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.

Requirements

- Ensure the arginine L-arginine hydrochloride 10% in 100-200mls (0.5g/kg – max dose 30 g) normal saline is prescribed and ordered from pharmacy prior to the patient's admission.
- 6 brown top serum tubes

Procedure

PATIENT PREPARATION

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

THE TEST

Insert IV cannulas in both right and left arms - one to give the infusion and one to take blood from.

Minutes	Procedure	Samples
-30	Insert an indwelling cannula into each arm 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 10% in 100-200mls normal saline over 30 minutes at a dose of 0.5g/kg (max 30g in 100-200mls Normal 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

Remember:

- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

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

Cortisol Day Curve on Metyrapone

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

Contraindications
None required.

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
- Insert *iv* cannula
- Note time and dose of medications.

TEST
Fill in Day Curve form on page 12

Time	Procedure	Sample
09:00	Take sample for **cortisol**	1 x brown top serum tube
12:00	Take sample for **cortisol**	1 x brown top serum tube
15:00	Take sample for **cortisol**	1 x brown top serum tube
18:00	Take sample for **cortisol**	1 x brown top serum tube
21:00	Take sample for **cortisol**	1 x brown top serum tube
24:00	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 referred for measurement of cortisol by mass spectrometry in these patients.

Remember:
- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

Day Curve Chart

This chart is to be used for any patient admitted for a day curve on PIU.

TO BE FILED IN THE PATIENT NOTES – DO NOT SEND TO THE LAB

DAY CURVE TYPE – please state e.g. Hydrocortisone, Metyrapone, Growth Hormone, 17OHP	

For the correct procedure please refer to the Protocols for Dynamic Function Test folder and follow the correct protocol for the day curve being carried out.

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.

Time	Sample Taken e.g. cortisol/GH	Results
ATTENTION: A pre-dose sample is always required :		
:		
:		
:		
:		
:		

Time	Medication Please note below the type and dose of medication against the time it was taken
:	
:	
:	
:	

Day Curve on Hydrocortisone

Indications:
Establishment of the 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
- 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.

TEST
***IMPORTANT:** A BASELINE SAMPLE SHOULD BE TAKEN IN THE MORNING **BEFORE** THE PATIENT TAKES THEIR MORNING DOSE OF HYDROCORTISONE.
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

Fill in Day Curve form on page 10

Time	Procedure	Samples
On patient Arrival	Take sample for **cortisol pre-dose**	1 x brown top serum tube
08:00	Patient should take normal morning dose of hydrocortisone	
08:30	Insert IV cannula	
09:00	Take sample for **cortisol**	1 x brown top serum tube
12:30	Take sample for **cortisol**	1 x brown top serum tube
14:00	Patient should take afternoon dose of hydrocortisone	Please note, this time may vary between patients
17:30	Take sample for **cortisol**	1 x brown top serum tube

Interpretation of results
09:00 cortisol should be in range of 133-537 nmol/L.
The 12:30 and 17:30 value should be >100 nmol/L.

Glucagon Stimulation Test

Principle
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 (09:00h level <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 should be stopped 24 hours before the test.
- Fast from midnight.
- Calculate glucagon dose: adults: 1 mg, (1.5mg if >90kg),

Remember:
- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

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

Cortisol Peak: >430nmol/L
Growth Hormone Peak: >6.7 µg/L.
Glucose Should show a transient fall followed by a rise.

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%. Only 4-8 % of normals will not show an adequate rise in GH: this is usually in patients over 50.

GnRH Test

Principle
GnRH (gonadotrophin releasing hormone) is a decapeptide secreted by the hypothalamus which stimulates the production and secretion of LH and FSH by the anterior pituitary

Indications
To diagnose hypothalamic-pituitary disease in precocious and delayed puberty in both sexes.

Side effects
GnRH may rarely cause nausea, headache and abdominal pain.

Requirements

- LH/FSH releasing hormone (GnRH) – 100 µg as i.v. bolus.
- 3 x brown top serum tubes

Procedure

PATIENT PREPARATION

- Admit the patient to the patient to the Programmed Investigation Unit on the day of the test
- No specific preparation is required

TEST

Time	Procedure	Sample
0	Take basal sample for LH and FSH. Then give GnRH 100µg as i.v bolus	1 x brown top serum tube (LH and FSH)
30	Take sample for LH and FSH	1 x brown top serum tube (LH and FSH)
60	Take sample for LH and FSH	1 x brown top serum tube (LH and FSH)

Interpretation of results

Normal Response:

LH	peak response 2-3x baseline
FSH	peak response 2-3x baseline

An inadequate response may be an early indication of hypopituitarism or delayed puberty. Gonadotrophic deficiency is diagnosed on the basal levels rather than the dynamic response.

Remember:

- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

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
- 5 x brown top serum tubes

Procedure
- Fill in the Day Curve Chart on Page 10

Protocol Time	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	
Base	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)
1	12:00	Take sample for Growth Hormone	1 x brown top serum
2	15:00	Take sample for Growth Hormone	1 x brown top serum
3	18:00	Take sample for Growth Hormone	1 x brown top serum

Interpretation of results

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

Remember:
- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

Growth Hormone Suppression Test

Principle

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® supplied by central stores 113ml or 75g anhydrous glucose in cold water plus 150ml cold water .
- 5 x yellow top fluoride EDTA tubes, 6 x brown top serum tubes
- Indwelling cannula.

Procedure

PATIENT PREPARATION

The patient should fast overnight (10-14 hours) and should rest throughout the test

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)

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.

GH may fail to suppress due to chronic renal failure, liver failure, active hepatitis, anorexia nervosa, malnutrition, hyperthyroidism, diabetes and 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)

HCG Stimulation Test

Principle
hCG is a double polypeptide hormone and shares a common subunit with LH. It stimulates testicular Leydig cells to secrete androgens via the LH receptors. A single injection of hCG is adequate as it has a long half life (2.5 days) and produces a progressive but modest rise in plasma testosterone for 72-120 hours.

Indications
- To detect functioning testicular tissue (e.g. in undescended testes or cryptorchidism).
- To define enzyme blocks in testosterone biosynthesis.
- In male delayed puberty and/or undescended testes

Side Effects
- Headaches
- Tiredness

Requirements
- 3000IU HCG
- 2 x brown top serum tubes

Procedure

Day	Procedure	Samples
1 (8-9am)	Take sample for **testosterone** (and androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected)	1 x brown top serum tube (testosterone, androstenedione and dihydrotestosterone)
1	Give 3000IU of HCG i.m.	
4	Take sample for **testosterone** (and androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected)	1 x brown top serum tube (testosterone, androstenedione and dihydrotestosterone)

Interpretation of results
A normal increment of testosterone after HCG is two-three fold the basal value.

If there is a defect in testosterone biosynthesis, there will be an absent testosterone response and an increase in testosterone precursors e.g. androstenedione response in the case of 17-ketosteroid reductase deficiency.

If DHT has been requested a normal testosterone:dihydrotestosterone is <17 before and after HCG stimulation in adult males.
In 5α- reductase deficiency the testosterone:dihydrotestosterone is <20 before HCG stimulation and >27 after stimulation.

High Dose Dexamethasone Suppression Test

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 (9am, then 3pm, 9pm, 3am, 9am, 3pm, 9pm, 3am) 2mg in adults.
- 2 x brown top serum tubes for cortisol
- 2 x orange top lithium heparin tubes for ACTH

Procedure

<u>PATIENT PREPARATION</u>

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

TEST

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 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.

Hypertonic Saline Infusion Test

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

Background information

This is a specialist investigation and should only be conducted after referral to Endocrinology and performed as an inpatient.

Principle

An increase in plasma 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. Plasma copeptin, the C-terminal glycoprotein of the AVP prohormone, is measured as a more stable marker of AVP secretion. Plasma copeptin is reported in relation to serum osmolality and assessed using a normogram. Plasma copeptin and AVP levels have been shown to agree well, see example on page 36.

Indications

To make a clear diagnosis of cranial diabetes insipidus (DI) in subjects with polyuria and normal serum osmolality.

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

- 7 x brown top serum sample tubes
- 7 x orange top lithium heparin tubes
- 5% saline

Procedure

PATIENT PREPARATION

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

TEST

Inform the Biochemistry laboratory, extension 64375 *[TGH 746 2494]*, that the samples for copeptin will be arriving so that samples can be processed promptly. Samples should arrive within 2hrs.

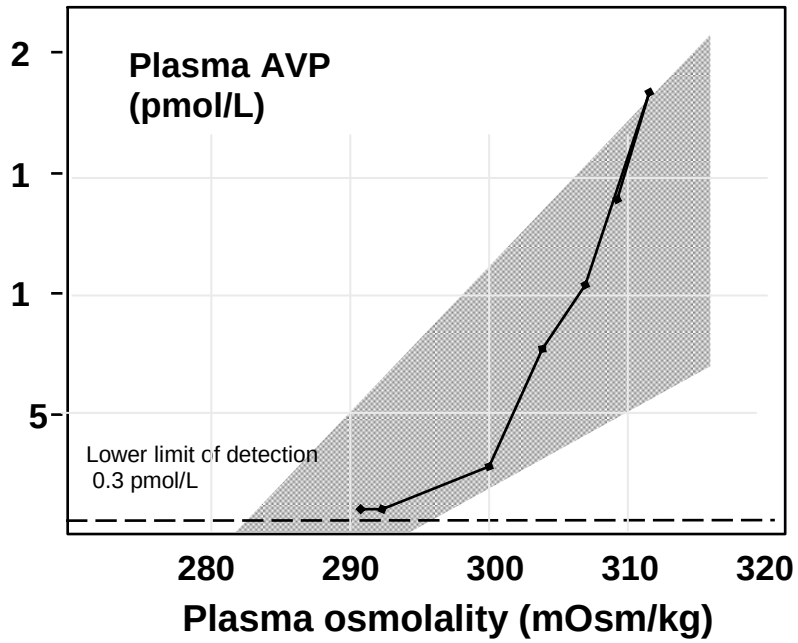
- A thirst chart should be completed throughout the test
- BP should be measured at regular intervals throughout the test

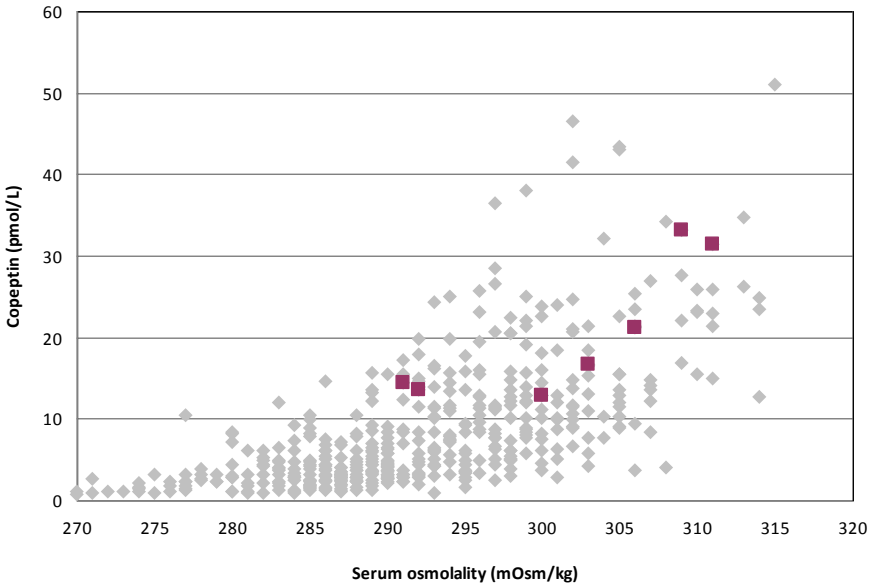
Time	Minutes	Procedure	Samples
08:45	-30	Basal Sample	1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
09:00	0	Infuse 5% saline 0.06ml/kg/min for 2 hours	1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
09:30	30		1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
10:00	60		1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
10:30	90		1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
11:00	120	STOP SALINE INFUSION	1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)
11:15	135		1 x brown top serum (osmolality) 1 x orange top Li Hep plasma (copeptin)

NOTE: **Please ensure that times are written clearly on the tubes. Samples should arrive in the laboratory within 2 hours of collection.**

Interpretation of results

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





Results that fall within the central area of the graph reflect a normal response. Points dropping to the right show decreased AVP/copeptin relative to normal osmoregulation (as would occur in cranial DI). [3]

Reference[3]: graphs provided as examples by the referral laboratory at Royal Victoria Hospital, Newcastle-upon-Tyne.

Insulin Tolerance Test
Blood Glucose/Hypoglycaemia Chart

Name: **Hosp No:**

Diagnosis: **ECG**

Wt in Kg:

Dose of Insulin given:

TIME (mins)	Test Strip Glucose	Symptoms
0		
30		
45		
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 :

Insulin Tolerance Test

Principle
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

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

This test is not advocated for use in Children (<16years old) – Please refer to Paediatric DFT protocols

Contraindications

- Age >60 years
- Ischaemic Heart Disease
- Epilepsy or unexplained blackouts
- Severe panhypopituitarism, hypoadrenalism (9:00 cortisol <100nmol/L)
- Glycogen Storage Disease
- Hypocalcaemia/Hypokalaemia

Precautions

- ECG must be normal
- Serum Cortisol must be >100nmol/L at 9am
- Thyroxine deficiency may reduce GH and cortisol response
- 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
  - Additional insulin may be required for acromegalic and diabetic patients if symptomatic or biochemical hypoglycaemia is not achieved after 60 minutes – consult with medical staff
- 50ml 50% dextrose available for immediate administration for hypoglycaemia.
- Indwelling cannula, 3 way tap.
- 100mg ampoule of hydrocortisone.
- Orange or blackcurrant juice (not sugar free)
- Hypoglycaemia symptom chart – see page 6
- 6 brown top serum tubes and six yellow fluoride EDTA tubes

Procedure

PATIENT PREPARATION

- Admit the patient to the Programmed Investigation Unit on the day before the test.
- Perform a 9 am serum cortisol, U and E and Bone Profile.
- Review by a doctor.
- Obtain informed consent from the patient
- Perform an ECG (which must be normal to proceed)
- Weigh the patient and document accurately in the medical notes, this is required to calculate the insulin dose required.
- Fast the patient from midnight.

TEST

This test is potentially dangerous.
A doctor or nurse must be in attendance at all times.
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 25ml of 50% dextrose i.v. 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 in hypoglycaemia chart. 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 in hypoglycaemia chart. 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 in hypoglycaemia chart. 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 in hypoglycaemia chart. Take a glucometer strip reading	1 x brown top serum (GH and cortisol) 1 x yellow top fluoride EDTA (glucose)

Remember:

- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**
- **If supplementary insulin has had to be given to achieve adequate hypoglycaemia, the test should be prolonged by 60 minutes**

AFTERCARE

Upon completion of test give patient a sugary drink and observe for 2 hours.
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.

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)

Ischaemic Forearm Test

Important:
Inform the Biochemistry Laboratory, extension 64375 *[TGH 746 2494]*, before performing the test and when samples are taken so that they can be processed in the laboratory immediately.

Principle

This test assesses the production of lactate by glycolysis and ammonia from AMP during anaerobic exercise muscle.

Indications

This test is useful in the differential diagnosis of muscle weaknesses, fatigue and cramps e.g McArdle's (myophosphorylase deficiency) or myoadenylate deaminase deficiency. In McArdle's disease glycogen breakdown to glucose and lactate is impaired.
These disorders should be considered in all patients who complain of muscle cramps and exercise intolerance.

Contraindications

The development of rhabdomyolysis has been reported in patients with underlying acquired (e.g. alcoholic or hypothyroid) or inherited myopathies who have been strenuously exercised.

Side Effects

This test is uncomfortable/painful to the patient with subjects complaining of pain and/or cramp.

Requirements

- 7 x pink top EDTA blood tubes
- 7 x yellow top fluoride EDTA tubes
- Ice for samples
- Sphygmomanometer cuff and bulb
- Spare sphygmomanometer bulb (for the patient to squeeze)

Procedure

PATIENT PREPARATION

- Patient should rest for 30 minutes prior to test
- Patients should not smoke on the morning before or during the test

PROCEDURE

NOTE: The patient's dominant arm should be chosen to perform the exercise. Blood samples should be taken from this arm throughout the test.

Minutes	Procedure	Samples
30 minutes pre test	Insert iv cannula into antecubital vein of the dominant arm	
Immediately pre test	Take pre-exercise samples	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
Exercise Test	Perform exercise procedure as stated below	
0	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
1	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
3	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
5	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
7	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)
10	Take samples for ammonia and lactate	1 x pink top EDTA (ammonia) 1 x yellow top fluoride EDTA (lactate)

Important: Samples should be batched together and sent to the laboratory immediately on ice. Inform Biochemistry laboratory, extension 64375 *[TGH 746 2494],* that the samples for ammonia and lactate will be arriving as they will have to be processed very quickly.

Exercise Procedure

1. Inflate sphygmomanometer cuff to 20mm above systolic pressure.
2. Ask patient to squeeze spare sphygmomanometer bulb once every second for one minute. Emphasise that it is important to squeeze the ball as energetically as possible.
   Note: Patients with muscle problems may not manage one minute due to severe pain and/or cramp. Stop the test if cramp ensues but collect blood samples at appropriate time.
3. Allow patient to rest for one minute with the cuff still inflated to allow lactate to leak out of the forearm muscles. (ischaemia)
4. Release the cuff and commence collection of blood samples as in the table – this is time 0.

Remember:

- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

Interpretation of Results

Normal Response
The lactate should rise in the first sample after ischaemic exercise to between 3-5 times the pre-exercise level and then gradually decline.
Maximal rise in ammonia to >70µmol/L

Disorders of glycogen mobilisation and glycolysis (McArdle's)
Little or no rise in lactate with a normal rise in ammonia.

Myoadenylate deaminase deficiency
Normal rise in lactate with absence of a rise in ammonia.

Note: A failure of both lactate and ammonia to rise suggests that the patient did not exercise adequately and the test should be repeated.

Lactose Tolerance Test

Principle

Lactose is broken down in the intestine by the enzyme lactase to galactose and glucose, which are then absorbed into the bloodstream from the intestine. In patients with lactose intolerance this enzyme is lacking and subsequently lactose is neither broken down nor absorbed in the intestine. Following ingestion of lactose-containing products the blood glucose fails to rise and lactose reaches the colon where it is utilised by colonic bacteria resulting in diarrhoea, gas and abdominal pain.

Indications

The lactose tolerance test is a test for diagnosing an intolerance of ingested lactose.

Contraindications

- Do not perform in patients with known glucose intolerance (including diabetes) as results cannot be interpreted.

Side Effects

- Diarrhoea
- Gas
- Abdominal Pain

Requirements

- 50g of lactose dissolved in 300ml water
- 5 x yellow top fluoride EDTA tubes

Procedure

PATIENT PREPARATION

- 3 days of unrestricted diet
- Fast from midnight (sips of water are permitted)
- Smoking should be avoided on the day of the test

TEST

Minutes	Procedure	Samples
0	Take basal sample for glucose Lactose solution should be drunk over 5 mins	1 x yellow top fluoride EDTA glucose
30	Take sample for glucose	1 x yellow top fluoride EDTA glucose
60	Take sample for glucose	1 x yellow top fluoride EDTA glucose
90	Take sample for glucose	1 x yellow top fluoride EDTA glucose
120	Take sample for glucose	1 x yellow top fluoride EDTA glucose

Interpretation of Results

A failure of blood glucose to rise by at least 1.0mmol/L and the spontaneous complaint of gastrointestinal symptoms are consistent with lactase deficiency. Failure of glucose to rise adequately may also be due to non-compliance by the patient, vomiting of the test dose or delayed gastric emptying.

Low Dose Dexamethasone Suppression Test

Indications

Screening test for Cushing's syndrome, especially if the result of the overnight suppression test is inconclusive.

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 (9am, then 3pm, 9pm, 3am, 9am, 3pm, 9pm, 3am **must adhere to the 6-hourly dosing frequency, especially important not to omit or delay the 3am 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

Procedure

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.

TEST

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 0.5mg dexamethasone p.o	
1500	Patient takes 0.5mg dexamethasone p.o	
2100	Patient takes 0.5mg dexamethasone p.o	
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	
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 2 is less than 50nmol/l the patient has shown suppression.

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.

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.

SENSITIVITY AND SPECIFICITY
Suppression in patients with Cushing's syndrome is rare (2-5%). 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.

Oral Glucose Tolerance Test

Principle

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

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

- This test is only necessary if fasting and/or random glucose measurements are equivocal i.e. 5.6 - <7.0 mmol/L.
- This test should NOT be performed in patients who fulfil the criteria for diabetes mellitus. These are:
  1) Two diagnostic glucose results on separate occasions; either fasting plasma glucose ≥7.0 mmol/L or random plasma glucose of ≥11.1 mmol/L.
  2) One diagnostic glucose result and clinical symptoms of diabetes e.g. polydipsia, polyuria, ketonuria and rapid weight loss.
- 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® supplied by central stores 113ml or 75g anhydrous glucose in cold water plus 150ml cold water .
- 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 for 10-14 hours 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.

TEST

This test should be performed in the morning. Patients should remain at rest during the test and should not be allowed to smoke.

Minutes	Procedure	Samples
0	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
0	Glucose solution/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	7.0 or greater	or	11.1 or greater
Gestational diabetes mellitus	>5.6	and	7.8 or greater

Ovarian cyclical functioning tracking

Indications

The investigation of menstrual disturbances and/or assessment of ovarian function.

Requirements

- 4 x brown top serum tubes (may need 2 further tubes if extended test)

Procedure

Take samples for LH, FSH and oestradiol and progesterone weekly throughout the cycle.

If the patient is menstruating, Day 1 is the first day of menstruation.

Patient is asked to record dates of first day of menstrual bleed before and after the tests.

Day	Procedure	Samples
1	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)
8	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)
15	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)
22	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)
May need extending to 6 weeks in some amenorrhoeic/oligomenorrhoeic patients		
29	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)
36	Take samples for **LH, FSH and Oestradiol and Progesterone**	1 x brown top serum (LH, FSH and Oestradiol, Progesterone)

Interpretation of results

Normal results should show rise and fall of FSH, LH and oestradiol within the follicular, mid-cycle and luteal phase reference ranges.

In patients with oligomenorrhoea and, more rarely, amenorrhoea, LH, FSH and oestradiol increments may not reach the reference range. This information may be helpful in determining the level of hypothalamic-pituitary-

ovarian axis function, for example in the assessment of the degree of development, suppression or recovery.

An FSH >30mU/L throughout the cycle is consistent with ovarian failure. All results should be considered along with clinical history.

Progesterone >15nmol/L indicates ovulation.

Overnight Dexamethasone Suppression Test (diagnosis of Cushing's)

Tests for screening patients for Cushing's syndrome include midnight and 9am salivary cortisol samples; collection of 3 consecutive 24 hour urine tests for Urine free cortisol; or an overnight dexamethasone suppression test.
Tubes for salivary cortisol estimation are available from the Biochemistry department.
Urine for estimation of 24 hour free cortisol excretion should be collected in a bottle without preservative. The patient should be issued with a request form, a 5 litre container for each test and an instruction sheet.

The patient's name, date of birth or record number should be written on the bottle. Ask the patient to write the collection time details on the bottle and to make sure that the request form is attached and to bring it to the outpatients or the Biochemistry department promptly.

Dexamethasone Suppression Test

Principle

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 takes 1 mg dexamethasone orally at 2300h and the following morning at exactly 0900h a blood sample is taken for serum cortisol.

1 brown top serum tube (cortisol)

Interpretation of results

A normal response is shown by suppression of 0900 h 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.

SENSITIVITY AND SPECIFICITY

If there is strong clinical or biochemical evidence for Cushing's syndrome, a formal 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%).
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Pentagastrin Stimulation Test

Principle
Calcitonin may be secreted by the C-cells of the thyroid gland. High levels may suggest medullary thyroid carcinoma. In very early disease (ie on screening for familial syndromes), levels may not be raised, but may be stimulated by pentagastrin. It is suggested that borderline baseline values are further investigated by stimulation**.**

Indications:

Screening for medullary cell carcinoma in MEN2 patients or their relatives.

Contraindications:

- Hypocalcaemia
- Hypertension
- Coronary Artery disease
- >60 years of age
- Pregnancy
- Asthma

Side Effects

- transient flushing
- nausea
- abdominal cramps
- Dizziness
- Bradycardia

Requirements

- Ensure pentagastrin is available from pharmacy**.** One Ampoule of pentagastrin (as 500µg for injection) is required.
- Insulin syringe, 5mL syringe
- 5mL vial of 0.9% saline
- Intravenous cannula, vacutainer luer lock adapter
- 4 brown top serum tubes
- Iced slurry for transport to the laboratory
- A fellow colleague to assist in the timing of the test

Procedure

PATIENT PREPARATION

WARNING: **Only perform this test under medical supervision**

- Admit the patient to the Programmed Investigation Unit.
- Review by a doctor.
- A light meal is allowed, but the patient should not have alcohol for 12hrs.
- Weigh the patient

TEST

Minutes	Procedure	Samples
	Insert an indwelling cannula	
0	Take basal sample for **calcitonin**.	1 x brown top serum tube On iced slurry
Draw up pentagastrin 0.5µg/kg into insulin syringe, add to 2ml saline in a 5ml syringe and inject i.v. over 15 seconds		
2	Take sample for **calcitonin**.	1 x brown top serum tube On iced slurry
5	Take sample for **calcitonin**	1 x brown top serum tube On iced slurry
10	Take sample for **calcitonin**	1 x brown top serum tube On iced slurry

NB: Calcitonin is unstable ex-vivo. **Each sample must be cooled in iced slurry and delivered immediately to the laboratory**

Interpretation of results

A rise of <10ng/ml is seen in healthy patients.
A rise in plasma calcitonin above 100 ng/ml is suggestive of C-cell hyperplasia.
A rise of between 10 and 100 ng/ml are equivocal and may require further investigation depending on the clinical picture.

Prolonged Oral Glucose Tolerance Test

Indications
This test is an extension of the standard oral glucose tolerance test in cases of suspected reactive hypoglycaemia.

Contra-indications
None

Side Effects
None

Requirements
- POLYCAL® supplied by central stores 113ml or 75g anhydrous glucose in cold water plus 150ml cold water .
- 10 x yellow top fluoride EDTA tubes

Procedure
PATIENT PREPARATION
- Fast from Midnight (sips of water are permitted)
- Smoking should be avoided on the day of the test
- Any regular medication should be taken as normal

TEST Record any symptoms of hypoglycaemia in the patient notes.

Minutes	Procedure	Samples
0	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
0	Glucose solution/Polycal® should be drunk over 5 minutes followed by the water	
30	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
60	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
90	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
120	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
150	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
180	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
240	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
270	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)
300	Take sample for **glucose**	1 x yellow top fluoride EDTA (glucose)

Interpretation of results A glucose result <3 mmol/L is consistent with reactive hypoglycaemia and requires follow up.

Renin and Aldosterone Studies

Principle

The renin-aldosterone axis is primarily regulated by renal blood flow. Subjects under investigation should, therefore, not be taking any drugs that interfere with fluid balance or potassium. Bethanidine, Doxazosin or Prazosin do not interfere with fluid balance or potassium and those subjects requiring hypotensive therapy should ideally be transferred to one of these agents. Secondly, it is essential that subjects should be normally hydrated and have an adequate oral intake of sodium. Hypokalaemia must be avoided since it suppresses aldosterone secretion. It is important to note that the effect of increasing oral sodium will be to considerably increase urinary potassium excretion.
Aldosterone renin ratio (ARR) is most sensitive when used in patients from whom samples are collected in the morning after patients have been out of bed for at least 2 hours and after they have been seated for 15 minutes.

Indications

- Accelerated hypertension.
- Drug resistant hypertension
- Hypertension and adrenal incidentaloma
- Hypertension with hypokalaemia, spontaneous or easily provoked, i.e. by diuretics or sodium loading – consider if plasma potassium is <3.5mmol/L. As the treatment of hyperaldosteronism is far more effective in correcting hypokalaemia rather than the hypertension extensive investigation in normokalaemic patients is not justified.

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Contraindications

None

Side Effects

None

Requirements

- 1 x pink top EDTA tube
- 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

- Give potassium replacement (Slow K tabs) sufficient to raise plasma potassium into the reference range (3.5-5.5 mmol/L). Replacement should be stopped on the day of the test.
- The patient must be sodium replete.
- Spironolactone and Eplerenone must be stopped for 6 weeks to be certain that any elevation in plasma renin activity is not due their antagonistic action on aldosterone receptors.
- Ideally all interfering drugs should be stopped, but if this is impractical, a best pragmatic approach is to stop ACE inhibitors, beta-blockers for 2 weeks and to avoid Ca-channel blockers on the day of the test.
- The optimal approach is to use either Bethanidine, Doxazosin, Hydralazine, Terazosin or Prazosin which do not appear to affect the renin-aldosterone axis.

Drug	Physiological effect	Time to remove interference
ACE inhibitors	increase PRA & reduce aldosterone	2 weeks
beta-blockers	reduce PRA more than aldosterone	2 weeks
Calcium channel blockers	reduce aldosterone and stimulate renin production	2 weeks
Diuretics	increase PRA and aldosterone	2 weeks
hypokalaemia	inhibits aldosterone secretion	
NSAIDs	retain sodium & reduce PRA, ? effect on aldosterone	2 weeks
Oestradiol	increase renin substrate	6 weeks
Spironolactone	increase PRA, variable effect on aldosterone	6 weeks

TEST

This test should ideally take place at 8am when aldosterone is highest.

Minutes	Procedure	Samples
0	The patient should remain seated for 10 mins prior to venepuncture	
10	Take sample for **Plasma renin activity and aldosterone**	1x pink top EDTA tube **NOT ON ICE**

Important:
This sample requires separating in the lab within 30 minutes of taking – please send ASAP – not on ice.

Interpretation of results

An aldosterone:renin ratio (ARR)>1000, when aldosterone measured in pmol/L and plasma renin activity in nmol/L/hr, indicates primary hyperaldosteronism[1]. The diagnosis should be confirmed by completing a saline infusion test, see page 15.
Some patients with renal disease may give similar results.

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

Saline Infusion Test for Hyperaldosteronism

Principle

The principle of this test is that 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 confirmation of primary aldosteronism. Patients should already have been screened with a random Aldosterone:Renin Ratio (see Renin and Aldosterone studies).

This should have found to have an elevated value (Aldosterone:Renin Ratio > 1000 and an aldosterone >250 pmol/L). This screening test should be done following the cessation of beta blockers, diuretics, calcium channel blockers, ACE-inhibitors and angiotensin II blockers as outlined on page 14.

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
- 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 spironolactone and eplerenone for 6 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 e.g. doxazosin
- Ensure plasma K in normal range (ideally >4) prior to performing test
- Examine patient for signs of cardiac failure.
- Patients stay in the recumbent position for at least 1 hour before test begins

TEST

Position patient in recumbent position prior to commencing procedure and sampling. The patient should remain recumbent throughout test.

Blood pressure, oxygen saturation and heart rate are monitored 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 15 minutes	
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** 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** 1 x brown top serum (U and E's)

Important:
This sample requires separating in the lab within 30 minutes of taking – please send ASAP – Not on ice.

Interpretation of results[1]

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

Post-infusion plasma aldosterone levels <140 pmol/L make the diagnosis of primary hyperaldosteronism unlikely, and levels >280pmol/ L are a very probable sign of primary hyperaldosteronism. Values between 140 – 280 pmol/L are indeterminate.

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

Short Synacthen Test

Principle
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
The investigation of adrenal insufficiency.

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

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.

Requirements
- 250 µg Synacthen (1 vial)
- 2x brown top serum tubes

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 then administer 250µg Synacthen IV	1 x brown top serum (cortisol)
30	Take 3ml blood for Cortisol	1 x brown top serum (cortisol)

Interpretation of results

Adrenal insufficiency is excluded by a 30 min value > 430 nmol/L.
For patients on glucocorticoid replacement interpretation of the response is not straightforward and depends on the duration, and dose of glucocorticoid treatment received.

Remember:
- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

Synacthen Stimulation of 17- Hydroxy Progesterone

Principle
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 17 OH-progesterone can be detected.

Indications
This is performed for the investigation of congenital adrenal hyperplasia (CAH) in children and adults.

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.

Requirements
- 2 x brown top serum tubes
- 250 microgram Synacthen (1 vial)

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	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: A normal response is <20nmol/L at 60 mins.
A level >30nmol/L is consistent with a diagnosis of CAH

Remember:
- **Clearly label samples with patient details and times**
- **Use the specific DFT protocol request forms**
- **Send all samples together to lab**

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 5%, or if serum osmolality rises above 300 mOsm/kg

Weight patient: **kg** **Calculate weight minus 5%:** **kg**

Time						
0 Hrs	:	**Take sample for urine and serum osmolality and send to the lab.**				
		Weight kg Loss >5%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes / No	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+3 Hrs	:	**Take sample for urine and serum osmolality and send to the lab.**				
		Weight kg Loss >5% Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes / No	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+5 Hrs	:	**Take sample for urine and serum osmolality and send to the lab.**				
		Weight kg Loss>5%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes / No	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+6 Hrs	:	**Take sample for urine and serum osmolality and send to the lab.**				
		Weight kg Loss >5%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes / No	Urine Osmolality **mOsm/kg**	**Urine Volume ml**	**BP mmHg**
+7 Hrs	:	**Take sample for urine and serum osmolality and send to the lab.**				
		Weight kg Loss >5%? Yes/No	**SerumOsmolality mOsm/kg** >300 mOsm/kg? Yes / No	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 i/m** 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	

Water Deprivation Test

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

Important:
Inform the Biochemistry laboratory, extension 64375 *[Trafford Biochemistry Lab Tel: 746 2494]*, at least 1 day in advance of performing this procedure so that samples can be processed efficiently.

Principle
Water restriction in the normal individual results in secretion of 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 (cranial 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 cranial or nephrogenic diabetes insipidus and primary polydipsia.

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

Other causes of polydipsia and polyuria:
- Diabetes Mellitus
- Hypoadrenalism
- Hypercalcaemia
- Hypokalaemia
- Hypothyroidism
- Urinary Infections
- Chronic kidney disease
- Therapy with Carbamazepine, Chlorpropamide, Lithium Therapy

Precautions
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.

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

Procedure
PATIENT PREPARATION:

Patient should be admitted to PIU on the day before the test.

1. If the patient is on DDAVP, this is discontinued 24hrs 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. Inform the laboratory 64375 *[TGH 746 2494]* the day before commencing the test

4. If indicated give normal steroid and/or thyroid hormone replacement before the test.

5. Tea, coffee, alcohol and tobacco are specifically excluded after midnight on the day of the test and during the test because they directly stimulate (vagus) the secretion of AVP independently of the osmoreceptors.

6. Patient is allowed to drink freely until the start of the test i.e. 08.00h.

7. A light breakfast is permitted before test commences e.g. 07.00h.

THE TEST

1. Print out the Water Deprivation test template on page 33 and fill in.

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 95% of their weight. Begin the fluid balance chart. Take urine and serum samples for osmolality

4. 12.00h, 14.00h, 15.00h, 16.00h the patient should be weighed and samples taken for serum and urine osmolality 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 >5% 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 (0.3µg in Children) i.m. at 17.00h and allow food and fluids.

Do not allow patient to drink >500ml for 8 hours after DDAVP administration as the patient will be at risk of developing profound hyponatraemia

6. Check urine and serum 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**
It may be sensible to keep them in hospital over night for monitoring

Interpretation of results

Post-Dehydration Osmolality (mOsm/kg)		Post DDAVP osmolality (mOsm/kg)	Diagnosis
Serum	**Urine**	**Urine**	
283-293	>750	>750	Normal
>293	<300	<300	Nephrogenic DI
>293	<300	>750	Cranial DI
<293	300-750	<750	Primary Polydipsia

n.b. : chronic primary polydipsia can dissipate the renal medullary osmotic gradient, thereby reducing the renal response to endogenous and exogenous AVP. In severe cranial DI, maximal urinary concentration may be achieved only after repeated DDAVP.

EQUIVOCAL RESULTS

May be due to partial DI or patient drinking during the test. In these cases the test can be repeated fasting the patient from midnight the night before the test.

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

If results are equivocal and there remains clinical suspicion of DI then proceed to hypertonic saline infusion test.

SENSITIVITY AND SPECIFICITY

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