Title: Dynamic Function Tests – Clinical Procedures

<table>
<thead>
<tr>
<th>Version</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Original Issue</td>
<td>15 May 2013</td>
</tr>
<tr>
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<td>All staff</td>
</tr>
<tr>
<td>Copy</td>
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</tr>
</tbody>
</table>
| Location of Copies | 1. Pathology Intranet site  
2. Medical Day unit  
3. Q-Pulse |

Important information:

All dynamic function tests with the exception of the short synacthen test and oral glucose tolerance test should be discussed with an Endocrinologist prior to performing.

Please note that some of these procedures include reference to patients <16 years of age. In children, all dynamic function tests should be authorised by the Paediatric Endocrinologist who may decide to liaise with the team at the Royal Victoria Infirmary in Newcastle.
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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
Screening test for adrenal insufficiency

Contraindications
Synacthen or Synacthen Depot is contra-indicated in patients with allergic disorders (e.g. asthma).

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

Precautions
- Ensure TSH and FT4 normal before commencing test.
- 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.
- Baseline and incremental cortisol values do NOT apply to women taking combined oral contraceptives, oestrogen HRT or to pregnant women. Combined OCP and HRT should be stopped for 6 weeks prior to the test.

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)
- 2 yellow top serum tubes

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

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take 3ml blood for Cortisol and then administer 250µg Synacthen i/m</td>
<td>1 x yellow top serum (cortisol)</td>
</tr>
<tr>
<td>30</td>
<td>Take 3ml blood for Cortisol</td>
<td>1 x yellow top serum (cortisol)</td>
</tr>
</tbody>
</table>

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

Adrenal insufficiency is excluded by a 30/60 min value > 550 nmol/L.

Patients with primary or secondary adrenocortical insufficiency fail to meet these criteria. These cases may require follow up with the three day Synacthen test and ACTH determinations.

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

The response to synacthen is not affected by obesity.

There is no difference in cortisol response between i.v and i.m administration.

Three day synacthen test

The short synacthen test may be followed by a 24 hour depot synacthen test, if the 60 minute result is equivocal. 1 mg depot synacthen is administered i.m. Samples for cortisol analysis should be taken at 0, 1, 2, 4, 8 and 24 hours.

<table>
<thead>
<tr>
<th>Time</th>
<th>95% confidence interval cortisol responses in normal subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1h</td>
<td>605 – 1265 nmol/L</td>
</tr>
<tr>
<td>2h</td>
<td>750 – 1520 nmol/L</td>
</tr>
<tr>
<td>4h</td>
<td>960 – 1650 nmol/L</td>
</tr>
<tr>
<td>8h</td>
<td>1025 – 1600 nmol/L</td>
</tr>
<tr>
<td>24h</td>
<td>610 – 1500 nmol/L</td>
</tr>
</tbody>
</table>

A gradual rise with a peak cortisol response at 4 – 8 hours occurs in normal subjects. Failure to respond, or initial unsustained response at 60 min, indicates primary adrenal failure.

A response rising to a peak at 24 hours occurs in secondary adrenal failure i.e. due to pituitary failure or prolonged corticosteroid therapy. Some cases of long-standing adrenal atrophy will not respond even after 24 hours and may require several daily doses of depot Synacthen before an adrenal response is seen. These cases are usually identified by measuring plasma ACTH.
Paediatric Protocol

Low Dose Synacthen Test

Drugs given and dose:-

Synacthen 0.5 mcg / 1.73m² i.v.

Test must be carried out first thing in the morning, preferably at 8am and no later than 9am. Measure weight and height and then calculate the surface area.

Prepare Synacthen as follows:

- Inject 1ml of synacthen (250 mcg) into 500mls of normal saline.
- Mix thoroughly
- Withdraw 1ml of mixture (0.5mcg) and dilute to 10mls with normal saline
- Dose = 10 x (Surface Area / 1.73) mls

Insert cannula and withdraw first blood samples at baseline (0 mins as listed)

Then give calculated dose of Synacthen i.v. Further samples to be taken at 30 and 60 minutes.

The child can eat and drink as they wish and can go home after the investigations are finished.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take 3ml blood for Cortisol and 3ml blood for ACTH and then administer Synacthen i/m</td>
<td>1 x yellow top serum (cortisol) 1 x purple top EDTA (ACTH)</td>
</tr>
<tr>
<td>30</td>
<td>Take 3ml blood for Cortisol</td>
<td>1 x yellow top serum (cortisol)</td>
</tr>
<tr>
<td>60</td>
<td>Take 3 ml blood for Cortisol</td>
<td>1 x yellow top serum (cortisol)</td>
</tr>
</tbody>
</table>
Insulin Tolerance Test

If you are considering this test in a patient <16 years please consult with the Paediatric Endocrinology Consultant as the arginine stimulation test or glucagon suppression test may be more appropriate.

This test is not without danger and must be performed with every precaution and under constant medical supervision

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.

Indications

- Diagnosis of secondary adrenal failure.
- Diagnosis of growth hormone deficiency.
- Differentiation of Cushing’s Syndrome from pseudo-Cushing’s e.g. depression, alcohol excess

Contraindications

- Age >60 years – This test may be considered in those > 60 years but testing must be approved by a Consultant Endocrinologist.
- Age <2 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 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 – page 8
- Six yellow top serum tubes and six grey top fluoride oxalate tubes

An indwelling line must be in place for sampling and intravenous injection of glucose if necessary.

vi. A carbohydrate meal should be given at the end of the test.

vii. Inform the laboratory about the test. Stress that the glucose assays must be performed without delay throughout the test and the results must be phoned to the ward/clinic.

Procedure

PATIENT PREPARATION

- Admit the patient to your investigation unit, 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. If feasible continue with blood sampling as adequate pituitary stimulation will have occurred.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Insert iv cannula</td>
<td>1 x yellow top serum (GH and cortisol) 1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>30</td>
<td>Take samples for <em>GH, cortisol and glucose</em>  Observe symptoms and record in hypoglycaemia chart. Take a glucometer strip reading</td>
<td>1 x yellow top serum (GH and cortisol) 1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>60</td>
<td>Take samples for <em>GH, cortisol and glucose</em>  Observe symptoms and record in hypoglycaemia chart. Take a glucometer strip reading</td>
<td>1 x yellow top serum (GH and cortisol) 1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>90</td>
<td>Take samples for <em>GH, cortisol and glucose</em>  Observe symptoms and record in hypoglycaemia chart. Take a glucometer strip reading</td>
<td>1 x yellow top serum (GH and cortisol) 1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>120</td>
<td>Take samples for <em>GH, cortisol and glucose</em>  Observe symptoms and record in hypoglycaemia chart. Take a glucometer strip reading</td>
<td>1 x yellow top serum (GH and cortisol) 1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
</tbody>
</table>
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.

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

<table>
<thead>
<tr>
<th>Glucose</th>
<th>&lt;2.2mmol/L measured by the laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>Incremental rise of &gt;170nmol/L and to 550nmol/L</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>&gt;6.7 µg/L</td>
</tr>
</tbody>
</table>

Peak cortisol values of 300-550nmol/L mean the patient may not require full steroid replacement but may need cover during major illnesses and surgery. Such patients should carry a "steroid card" and the ITT or an SST should be repeated in 6-12 months.

For Cushing's syndrome - there is a rise in cortisol of less than 170nmol/L above the basal cortisol level. Patients that are depressed or alcoholic may show a normal rise.

Growth hormone deficiency of sufficient severity for GH replacement to have been shown 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)
**Insulin Tolerance Test**  
**Blood Glucose/Hypoglycaemia Chart**

Name: ___________________________  
Hosp No: _________________________

Diagnosis: _______________________

ECG

Wt in Kg:

Dose of Insulin given:

<table>
<thead>
<tr>
<th>TIME (mins)</th>
<th>Test Strip Glucose</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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 grey top fluoride oxalate tubes
- 6 yellow 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)
- Dose for children: 100ug/kg (max 1 mg) – refer to paediatric protocol

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

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>Insert an indwelling cannula</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Take basal samples for glucose, cortisol, TSH and FT4</td>
<td>1 x yellow top serum (GH and cortisol, TSH and FT4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>0</td>
<td>Give the glucagon im</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>120</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>150</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>180</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>210</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>240</td>
<td>Take samples for glucose, cortisol and GH.</td>
<td>1 x yellow top serum (GH and cortisol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x grey top fluoride oxalate (glucose)</td>
</tr>
</tbody>
</table>

Interpretation of results

- **Cortisol**: Incremental rise of >200nmol/L and to 550nmol/L
- **Growth Hormone**: Adults: > 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% (but only 8% of normal will neither show a peak value of 550nmol or a rise of 170nmol/L). Only 4-8% of normal will not show an adequate rise in GH: this is usually in patients over 50.

**Paediatric protocol – use under the supervision of a paediatric consultant only**

**Patient preparation:**
- As stated on page 9
- Have rescue medicine prepared (2mls/kg Polycal, 10% dextrose and 100 mg hydrocortisone)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>Insert an indwelling cannula</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Take basal samples for <strong>glucose, cortisol, GH, TSH and FT4</strong></td>
<td>1 x yellow top serum (GH and cortisol, TSH and FT4) 1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>0</td>
<td>Give the glucagon im</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
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<tr>
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<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
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<tr>
<td>120</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
<td>1 x yellow top serum (GH and cortisol) 1 x grey top fluoride oxalate (glucose)</td>
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<tr>
<td>150</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
<td>1 x yellow top serum (GH and cortisol) 1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>180</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
<td>1 x yellow top serum (GH and cortisol) 1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>210</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
<td>1 x yellow top serum (GH and cortisol) 1 x grey top fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>240</td>
<td>Take samples for <strong>glucose, cortisol and GH.</strong></td>
<td>1 x yellow top serum (GH and cortisol) 1 x grey top fluoride oxalate (glucose)</td>
</tr>
</tbody>
</table>
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
- Yellow 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.
- Note the weight, abdominal girth, hip girth and BP (supine and standing) in patient notes.

*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
TEST

Fill in Day Curve form on page 14

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>On patient Arrival</td>
<td>Take sample for cortisol pre-dose</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>08:00</td>
<td>Patient should take normal morning dose of hydrocortisone</td>
<td></td>
</tr>
<tr>
<td>08:30</td>
<td>Insert IV cannula</td>
<td></td>
</tr>
<tr>
<td>09:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>12:30</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>14:00</td>
<td>Patient should take afternoon dose of hydrocortisone</td>
<td>Please note, this time may vary between patients</td>
</tr>
<tr>
<td>17:30</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
</tbody>
</table>

**Interpretation of results**

09:00 cortisol should be in range of 200-650 nmol/L. The 12:30 and 17:30 value should be >100 nmol/L.
Day Curve Chart

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

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

<table>
<thead>
<tr>
<th>DAY CURVE TYPE – please state</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. Hydrocortisone, Metyrapone, Growth Hormone, 17OHP</td>
<td></td>
</tr>
</tbody>
</table>

Please 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.

<table>
<thead>
<tr>
<th>Time</th>
<th>Sample Taken e.g. cortisol/GH</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATTENTION: A pre-dose sample is always required</td>
<td></td>
</tr>
<tr>
<td></td>
<td>:</td>
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<table>
<thead>
<tr>
<th>Time</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Please note below the type and dose of medication against the time it was taken</td>
</tr>
<tr>
<td></td>
<td>:</td>
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<td></td>
<td>:</td>
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<td>:</td>
</tr>
</tbody>
</table>
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 14

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>12:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>15:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>18:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>21:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>24:00</td>
<td>Take sample for cortisol</td>
<td>1 x yellow top serum tube</td>
</tr>
</tbody>
</table>

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 precursors accumulate in patients on metyrapone and they cross-react in the cortisol assays, therefore the target range should be interpreted in light of this 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
Renin and Aldosterone Studies

Principle

The renin-alderosterone 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. Neither bethanidine nor Doxazosin or Prazosin interfere and those subjects requiring hypotensive therapy should ideally be transferred to one of these agents.

Secondly, it is essential that subject 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.

Contraindications

None

Side Effects

None

Requirements

- 2 x Purple top EDTA tubes
- Blood samples should be taken immediately (within 20 minutes) to the laboratory but not on ice as PRA (plasma renin activity) is measured by the activity of renin and at 4°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 to its inhibition of aldosterone.
- 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 as none appear to affect the renin-aldosterone axis.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Physiological effect</th>
<th>Time to remove interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td>increase PRA &amp; reduce aldosterone</td>
<td>2 weeks</td>
</tr>
<tr>
<td>beta-blockers</td>
<td>reduce PRA more than aldosterone</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>reduce aldosterone and stimulate renin production</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Diuretics</td>
<td>increase PRA and aldosterone</td>
<td>2 weeks</td>
</tr>
<tr>
<td>hypokalaemia</td>
<td>inhibits aldosterone secretion</td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>retain sodium &amp; reduce PRA, ? effect on aldosterone</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>increase renin substrate</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>increase PRA, variable effect on aldosterone</td>
<td>6 weeks</td>
</tr>
</tbody>
</table>

**TEST**

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

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>The patient should remain seated for 15 mins prior to venepuncture</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Take sample for <strong>Plasma renin activity and aldosterone</strong></td>
<td>2x Purple top EDTA samples, <strong>NOT ON ICE</strong></td>
</tr>
</tbody>
</table>

**Important:**

This sample requires separating in the lab. Please send ASAP – not on ice.
Interpretation of results

An aldosterone:renin ratio (ARR) >1000, when aldosterone pmol/L and plasma renin activity nmol/L/hr, indicates primary hyperaldosteronism. The diagnosis should be confirmed by completing a saline infusion test.

Some patients with renal disease may give similar results.

Reference: 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. Bethanidine nor Doxazosin or Prazosin interfere and those subjects requiring hypotensive therapy should ideally be transferred to one of these agents.

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

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

Requirements
- 2L 0.9% saline for IV administration
- infusion pump/giving set
- 2 indwelling catheters
- 4 Purple top EDTA tubes for plasma renin and aldosterone sampling.

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.*

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15</td>
<td>Site indwelling cannula for administration of 0.9% Saline infusion and cannula in opposite arm for blood sampling and leave for 15 minutes</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Take sample for aldosterone, Plasma renin activity and U + E’s</td>
<td>2 x Purple top EDTA (PRA and aldosterone)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x Yellow top serum (U + E’s)</td>
</tr>
<tr>
<td>0</td>
<td>Commence Infusion of 2L 0.9% saline over 4 hours</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>STOP INFUSION</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>Take sample for Aldosterone, Plasma renin activity and U + E’s</td>
<td>2 x Purple top EDTA (PRA and aldosterone)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x Yellow top serum (U + E’s)</td>
</tr>
</tbody>
</table>

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

**Interpretation of results**
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: Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline.
Synacthen Stimulation of 17-Hydroxyprogesterone – Adults only

**Principle**
Adrenal glucocorticoid secretion is controlled by adrenocorticotropic 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 adults.

**Contraindications**
The Synacthen test gives unreliable results within 2 weeks of pituitary surgery. In children this test should be done in collaboration with the Newcastle paediatric team.

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

**Requirements**
- 2 yellow 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.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take sample for <strong>Cortisol and 17 OHP</strong> and then administer 250µg Synacthen i/m</td>
<td>2 x yellow top serum tube (cortisol and 17OHP)</td>
</tr>
<tr>
<td>30</td>
<td>Take sample for <strong>Cortisol and 17 OHP</strong></td>
<td>2 x yellow top serum tube (cortisol and 17OHP)</td>
</tr>
<tr>
<td>60</td>
<td>Take sample for <strong>Cortisol and 17 OHP</strong></td>
<td>2 x yellow top serum tube (cortisol and 17OHP)</td>
</tr>
</tbody>
</table>

**Interpretation of results**
- **Cortisol**: Normal response at 30 mins: Incremental rise of >200nmol/L to a concentration of 550nmol/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
17-Hydroxyprogesterone day curve

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

Contraindications:
None

Requirements
- 6 x yellow top serum tubes for 17OHP measurement
- Cannula
- Day Curve form on Page 14

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 14**

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>12:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>15:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>18:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>21:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>24:00</td>
<td>Take sample for 17OHP</td>
<td>1 x yellow top serum tube</td>
</tr>
</tbody>
</table>

**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
GnRH Test

Principle
GnRH (gonadotropin 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 yellow top serum tubes.

Procedure

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

TEST

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take basal sample for LH and FSH. Then give GnRH 100µg as i.v bolus</td>
<td>1 x yellow top serum tube (LH and FSH)</td>
</tr>
<tr>
<td>30</td>
<td>Take sample for LH and FSH</td>
<td>1 x yellow top serum tube (LH and FSH)</td>
</tr>
<tr>
<td>60</td>
<td>Take sample for LH and FSH</td>
<td>1 x yellow top serum tube (LH and FSH)</td>
</tr>
</tbody>
</table>

Interpretation of results

Normal Response:
- LH: 2-3x baseline
- FSH: 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
**Overnight Dexamethasone Suppression Test**

**Principle**

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

**Indications**

This should be the first line screening test for all subjects suspected of having 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.
- For children, please liaise with Newcastle RVI paediatric team regarding doses.

**Side Effects**

None

**Requirements**

- 1 mg dexamethasone tablet
- 1 yellow top serum tube

**Procedure**

The patient takes 1 mg dexamethasone orally at 2300h and the following morning at exactly 0900h a yellow top blood sample is taken for plasma 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.

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%).
Low Dose Dexamethasone suppression test (LDSST)

Indications
Screening test for Cushing’s syndrome, especially if the result of the overnight suppression test contradicts other investigations.

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.
- For children, please liaise with Newcastle RVI paediatric team regarding doses.

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, children 20µg/kg.
- 2 x yellow top serum tubes for cortisol
- 2 x Purple top EDTA tubes for ACTH which must be sent on ice.

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 and CBG might be helpful in this circumstance.
TEST

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
</table>
| 0845  | Take basal samples for **cortisol** and **ACTH** | 1 x yellow top serum tube (cortisol)  
1 x purple top EDTA (ACTH) – send on ice 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 yellow top serum tube (cortisol)  
1 x purple top EDTA (ACTH) – send on ice 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.
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; we rarely use this test now. If ACTH-dependent Cushing's syndrome has been diagnosed following a LDDST, patients can move straight to IPSS to 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.
- For children, please liaise with Newcastle RVI paediatric team regarding doses.

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 yellow top serum tubes for cortisol
- 2 x Purple top EDTA tubes for ACTH

Procedure

PATIENT PREPARATION

- Stop all oral oestrogen therapy 6 weeks prior to test. Again implants can cause problems.
- This is an inpatient test and should only be performed after at least 2 baseline values for 24 hour urinary free cortisol and 0900h cortisol and ACTH levels (see below).
TEST

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
</table>
| 0845  | Take basal samples for cortisol and ACTH      | 1 x yellow top serum tube (cortisol)  
|       |                                                | 1 x purple top EDTA (ACTH) send to the lab on ice 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 yellow top serum tube (cortisol)  
|       |                                                | 1 x purple top EDTA (ACTH) send to the lab on ice 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.
Oral Glucose Tolerance Test for diagnosis of Acromegaly

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

- POLYCAL® 113ml or 75g anhydrous glucose.
- If using POLYCAL make up to 200ml with cold water (i.e. add 87mls).
- If using anhydrous glucose dissolve in 300 ml warm water. Allow to cool.
- 5 X Grey top fluoride oxalate vacutainers, 6 x serum yellow top tubes
- Indwelling cannula.

Procedure

PATIENT PREPARATION

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

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
</table>
| 0       | Insert cannula and take samples for **growth hormone, glucose and IGF-1**  
Drink glucose solution/polycal within 5 minutes | 1 x grey top fluoride oxalate (glucose)  
1 x yellow top serum tube (growth hormone and IGF-1) |
| 30      | Take samples for **Growth Hormone and Glucose** | 1 x grey top fluoride oxalate (glucose)  
1 x yellow top serum tube (growth hormone) |
| 60      | Take samples for **Growth Hormone and Glucose** | 1 x grey top fluoride oxalate (glucose)  
1 x yellow top serum tube (growth hormone) |
| 90      | Take samples for **Growth Hormone and Glucose** | 1 x grey top fluoride oxalate (glucose)  
1 x yellow top serum tube (growth hormone) |
| 120     | Take samples for **Growth Hormone and Glucose** | 1 x grey top fluoride oxalate (glucose)  
1 x yellow top serum tube (growth hormone) |

**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 subjects will exhibit suppression of GH to <0.7 µg/L

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.
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 yellow top serum tubes

Procedure
- Fill in the Day Curve Chart on Page 14.

<table>
<thead>
<tr>
<th>Protocol Time</th>
<th>Time</th>
<th>Procedure</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>08:45</td>
<td>Insert cannula and allow the patient to rest for 15 mins so that stress does not interfere with the results</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>09:00</td>
<td>Take sample for Growth Hormone and IGF-1</td>
<td>1 x yellow top serum tube (GH and IGF-1)</td>
</tr>
<tr>
<td>1</td>
<td>12:00</td>
<td>Take sample for Growth Hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>2</td>
<td>15:00</td>
<td>Take sample for Growth Hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>3</td>
<td>18:00</td>
<td>Take sample for Growth Hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
</tbody>
</table>

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
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 yellow 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.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>Insert an indwelling cannula into each arm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allow the patient to rest for at least 30 mins.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Take basal sample for growth hormone.</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>0</td>
<td>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)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Take sample for growth hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>60</td>
<td>Take sample for growth hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>90</td>
<td>Take sample for growth hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
<tr>
<td>120</td>
<td>Take sample for growth hormone</td>
<td>1 x yellow top serum tube</td>
</tr>
</tbody>
</table>

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
**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 43695 DMH/32439 UHND, 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.

Requirements
- Accurate scales for weighing the patient
- Universal urine pots for urine osmolality
- Yellow 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 43695 (DMH)/32439 (UHND) 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. 08:00.
7. A light breakfast is permitted before test commences e.g. 07:00.

THE TEST

1. Print out the Water Deprivation test template on page 33 and fill in.
2. At 08:00 the patient should empty their bladder and this urine should be discarded
3. 09:00 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. At 12:00, 14:00, 15:00 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 i.m (0.3µg in Children) or 20µg intranasal at 1500 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.

<table>
<thead>
<tr>
<th>Interpretation of results</th>
<th>Post-Dehydration Osmolality (mOsm/kg)</th>
<th>Post DDAVP osmolality (mOsm/kg)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Urine</td>
<td>Urine</td>
<td></td>
</tr>
<tr>
<td>283-293</td>
<td>&gt;750</td>
<td>&gt;750</td>
<td>Normal</td>
</tr>
<tr>
<td>&gt;293</td>
<td>&lt;300</td>
<td>&lt;300</td>
<td>Nephrogenic DI</td>
</tr>
<tr>
<td>&gt;293</td>
<td>&lt;300</td>
<td>&gt;750</td>
<td>Cranial DI</td>
</tr>
<tr>
<td>&lt;293</td>
<td>300-750</td>
<td>&lt;750</td>
<td>Primary Polydipsia</td>
</tr>
</tbody>
</table>

NB: 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 remain 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).
# WATER DEPRIVATION TEST TEMPLATE

<table>
<thead>
<tr>
<th>Time</th>
<th>Take sample for urine and serum osmolality and send to the lab.</th>
<th>Weight kg</th>
<th>Serum Osmolality mOsm/kg</th>
<th>Urine Osmolality mOsm/kg</th>
<th>Urine Volume ml</th>
<th>BP mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Hrs</td>
<td>Weight kg</td>
<td>Serum Osmolality mOsm/kg</td>
<td>Urine Osmolality mOsm/kg</td>
<td>Urine Volume ml</td>
<td>BP mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss &gt;5%? Yes/No</td>
<td>&gt;300 mOsm/kg?</td>
<td>Yes / No</td>
<td>\</td>
<td>\</td>
<td></td>
</tr>
<tr>
<td>+3 Hrs</td>
<td>Weight kg</td>
<td>Serum Osmolality mOsm/kg</td>
<td>Urine Osmolality mOsm/kg</td>
<td>Urine Volume ml</td>
<td>BP mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss &gt;5%? Yes/No</td>
<td>&gt;300 mOsm/kg?</td>
<td>Yes / No</td>
<td>\</td>
<td>\</td>
<td></td>
</tr>
<tr>
<td>+5 Hrs</td>
<td>Weight kg</td>
<td>Serum Osmolality mOsm/kg</td>
<td>Urine Osmolality mOsm/kg</td>
<td>Urine Volume ml</td>
<td>BP mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss &gt;5%? Yes/No</td>
<td>&gt;300 mOsm/kg?</td>
<td>Yes / No</td>
<td>\</td>
<td>\</td>
<td></td>
</tr>
<tr>
<td>+7 Hrs</td>
<td>Weight kg</td>
<td>Serum Osmolality mOsm/kg</td>
<td>Urine Osmolality mOsm/kg</td>
<td>Urine Volume ml</td>
<td>BP mmHg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Loss &gt;5%? Yes/No</td>
<td>&gt;300 mOsm/kg?</td>
<td>Yes / No</td>
<td>\</td>
<td>\</td>
<td></td>
</tr>
</tbody>
</table>

If the urine osmolality is >750 mOSM/kg STOP the test.
If urine osmolality is <750 mOSM/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.

<table>
<thead>
<tr>
<th>DDAVP - Time administered -</th>
<th>Allow food and minimal fluids DO NOT allow excessive drinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 2 hrs</td>
<td>Collect urine for osmolality</td>
</tr>
<tr>
<td>+ 4 hrs</td>
<td>Collect urine for osmolality</td>
</tr>
</tbody>
</table>

The test must be stopped if weight loss exceeds 5%, or if serum osmolality rises above 300 mOsm/kg.

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

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 AVP level can be assessed against plasma osmolality and assessed for normality using the Cardiff Chart (Dr M Penney) page 37.

Indications
To make a clear diagnosis of cranial Diabetes Insipidus (DI) in subjects with polyuria and normal plasma 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 yellow top serum sample tubes
- 14 x Lithium Heparin green top tubes (laboratory can supply) on ice
- 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 that the samples for AVP will be arriving as they will have to be processed very quickly.

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

<table>
<thead>
<tr>
<th>Time</th>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:45</td>
<td>-30</td>
<td>Basal Sample</td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>09:00</td>
<td>0</td>
<td>Infuse 5% saline 0.06ml/kg/min for 2 hours</td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>09:30</td>
<td>30</td>
<td></td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>10:00</td>
<td>60</td>
<td></td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>10:30</td>
<td>90</td>
<td></td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>11:00</td>
<td>120</td>
<td>STOP SALINE INFUSION</td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
<tr>
<td>11:15</td>
<td>135</td>
<td></td>
<td>1 x yellow top serum (osmolality)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2x Li Hep Green top plasma on ice (AVP)</td>
</tr>
</tbody>
</table>

**NOTE:** Samples for AVP require rapid processing and should therefore be sent to the lab immediately as they are taken. Please ensure that times are written clearly on the tubes

**Interpretation of results**

Patients with primary polydipsia or nephrogenic DI have normal AVP release in response to the hyperosmolar state induced by the procedure. Patients with cranial DI have little or no rise in AVP.
Results that fall within the central area of the graph reflect a normal response. Points dropping to the right show decreased AVP relative to normal osmoregulation (as would occur in cranial DI) and points to the left indicate osmotically inappropriate levels (as would occur in non-osmotic stimuli or SIADH).
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.

Contra-indications

- This test is only necessary if fasting and/or random glucose measurements are equivocal i.e. between 5.6 and 6.9 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® 113ml or 75g anhydrous glucose
- If using POLYCAL make up to 200ml with cold water (i.e. add 87mls)
- If using anhydrous glucose dissolve in 300 ml warm water. Allow to cool
- Beaker from pharmacy
- 2 X Grey top fluoride oxalate vacutainers

For Children
- For children use only anhydrous glucose, 1.75 g/Kg up to a maximum of 75 g. Dissolve glucose in warm water, 100 ml for every 25 g glucose. Allow to cool.

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.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take sample for <strong>glucose</strong></td>
<td>1 x Grey Top Fluoride Oxalate (glucose)</td>
</tr>
<tr>
<td>0</td>
<td>Glucose solution/Polycal® made up with water should be drunk over 5 minutes. Give patient 100ml of water following drinking the Glucose/Polycal solution.</td>
<td></td>
</tr>
<tr>
<td>60 – children only</td>
<td><strong>IN CHILDREN ONLY</strong> – take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
<tr>
<td>120</td>
<td>Take sample for <strong>glucose</strong></td>
<td>1 x Grey Top Fluoride oxalate (glucose)</td>
</tr>
</tbody>
</table>

Interpretation of results

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 minute</td>
</tr>
<tr>
<td><strong>Non Diabetic</strong></td>
<td>&lt;6.1</td>
</tr>
<tr>
<td><strong>Impaired fasting glucose</strong></td>
<td>6.1 - 6.9</td>
</tr>
<tr>
<td><strong>Impaired glucose tolerance</strong></td>
<td>&lt;7.0</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>7.0 or greater</td>
</tr>
</tbody>
</table>
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® 113ml or 75g anhydrous glucose
- If using POLYCAL make up to 200ml with cold water (i.e. add 87mls)
- If using anhydrous glucose dissolve in 300 ml warm water. Allow to cool
- Beaker from pharmacy
- 10 x Grey Top Fluoride Oxalate 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.

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride Oxalate</td>
</tr>
<tr>
<td>0</td>
<td>Glucose solution/PolyCal® made up with water should be drunk over 5 minutes. Give the patient 100ml of water.</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>60</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>90</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>120</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>150</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>180</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>240</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>270</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
<tr>
<td>300</td>
<td>Take sample for glucose</td>
<td>1 x Grey Top Fluoride oxalate</td>
</tr>
</tbody>
</table>

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

Indications

For the diagnosis of Insulinoma.

Requirements

- Grey Top fluoride oxalate sample tubes for glucose
- Yellow top serum sample tubes for insulin and C-peptide
- Ice for transportation of samples.
- 50ml 50% dextrose available for immediate administration for hypoglycaemia

Procedure

- The onset of the fast is classed as the last intake of calories.
- Calorie free, caffeine free beverages only may be taken.
- 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.
- Bedside blood glucose monitoring should 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 blood glucose is found to be <3mmol/L then a plasma glucose sample (Grey top tube) accompanied by insulin and c-peptide samples (2 x yellow top tubes on ice) should be sent to the laboratory. **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.
<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0900</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1300</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1700</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>2100</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0100</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0500</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0900</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1300</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1700</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>2100</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0100</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0500</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>0900</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1300</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
<tr>
<td>1700</td>
<td>Perform a bedside blood glucose and take samples for laboratory glucose, c-peptide and insulin if the blood glucose is &lt;3.0mmol/L</td>
</tr>
</tbody>
</table>
If the blood glucose measured at any time point on the point of care device reads <3.0mmol/L, send a grey top sample for glucose and 2 yellow top serum samples immediately on ice for insulin and c-peptide analysis.

**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.
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 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 – In children, refer to a paediatric endocrinologist for dosing
- 2 x Yellow top serum tubes

Procedure

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Take sample for <strong>testosterone</strong> (and androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected)</td>
<td>1 x Yellow top serum (testosterone, androstenedione and dihydrotestosterone)</td>
</tr>
<tr>
<td>(8-9am)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Give 3000IU of HCG i.m.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Take sample for <strong>testosterone</strong> (and androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected)</td>
<td>1 x Yellow top serum (testosterone, androstenedione and dihydrotestosterone)</td>
</tr>
</tbody>
</table>

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 androstenedione response.

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.
Ovarian cyclical function tracking

Indications

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

Requirements

- 4 x Yellow top serum tubes

Procedure

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

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

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Take samples for LH, FSH and Oestradiol</td>
<td>1 x Yellow top serum (LH, FSH and Oestradiol)</td>
</tr>
<tr>
<td>8</td>
<td>Take samples for LH, FSH and Oestradiol</td>
<td>1 x Yellow top serum (LH, FSH and Oestradiol)</td>
</tr>
<tr>
<td>15</td>
<td>Take samples for LH, FSH and Oestradiol</td>
<td>1 x Yellow top serum (LH, FSH and Oestradiol)</td>
</tr>
<tr>
<td>22</td>
<td>Take samples for LH, FSH and Oestradiol</td>
<td>1 x Yellow top serum (LH, FSH and Oestradiol)</td>
</tr>
</tbody>
</table>

Interpretation of results

An FSH >30mU/L throughout the cycle is consistent with ovarian failure. All results should be considered along with clinical history.
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 (i.e. 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 yellow top serum tubes
- Ice 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
- Review by a doctor.
- A light meal is allowed, but the patient should not have alcohol for 12hrs.
- Weigh the patient

**TEST**

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Procedure</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Take basal sample for calcitonin.</td>
<td>1 x yellow top serum tube On ice</td>
</tr>
<tr>
<td></td>
<td>Insert an indwelling cannula</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Take sample for calcitonin.</td>
<td>1 x yellow top serum tube On ice</td>
</tr>
<tr>
<td>5</td>
<td>Take sample for calcitonin</td>
<td>1 x yellow top serum tube On ice</td>
</tr>
<tr>
<td>10</td>
<td>Take sample for calcitonin</td>
<td>1 x yellow top serum tube On ice</td>
</tr>
</tbody>
</table>

**NB:** Calcitonin is unstable ex-vivo. Each sample must be cooled in ice 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.