Clinical Guideline

Dynamic Function Tests for Use in Paediatric Endocrinology

Approved by the SPEG Guidelines Group
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NOTE
This guideline is not intended to be construed or to serve as a standard of care. Standards of care are determined on the basis of all clinical data available for an individual case and are subject to change as scientific knowledge and technology advance and patterns of care evolve. Adherence to guideline recommendations will not ensure a successful outcome in every case, nor should they be construed as including all proper methods of care or excluding other acceptable methods of care aimed at the same results. The ultimate judgement must be made by the appropriate healthcare professional(s) responsible for clinical decisions regarding a particular clinical procedure or treatment plan. This judgement should only be arrived at following discussion of the options with the patient, covering the diagnostic and treatment choices available. It is advised, however, that significant departures from the national guideline or any local guidelines derived from it should be fully documented in the patient’s case notes at the time the relevant decision is taken.
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Introduction

This handbook is for clinicians managing children with endocrine disorders in Scotland and sets out standard ways of performing dynamic function tests.

This is a consensus document of good practice and evidence where available.

The dynamic function test protocols described in this handbook apply to all tests that can be used in children to assess endocrine disorders.

Some of these tests should only be performed in centres equipped to perform them, and in accordance with the appropriate level of care that the centres are able to provide.

For each test there is a statement as to whether it can be performed in any centre, or only in a tertiary centre which regularly performs the test.

Who is this document for?

This document is intended for use by:

- Paediatric endocrinologists
- General paediatricians with an interest in paediatric endocrinology
- Paediatric endocrine nurses
- Paediatric nurses
- Clinical biochemists
- Paediatricians in training

The document is not intended for use in primary care.
GLUCOSE TOLERANCE TEST

Indications for procedure

1. **Establish a diagnosis of Diabetes Mellitus.**
   
   It is unnecessary if a child has symptoms of diabetes and either a random venous plasma laboratory glucose concentration of 11.1 mmol/L or higher, or a fasting concentration of 7.0 mmol/L or higher. [Definition, Diagnosis and Classification of Diabetes Mellitus. WHO criteria 1999].
   
   Glucose is measured at 0 min and 120 minutes only.

2. **Assess Insulin Resistance** (see insulin resistance section below).
   
   In addition to the standard procedure glucose and insulin are measured at 0, 30, 60, 90 and 120 minutes.

Preparations for the test

1. Do not perform glucose tolerance tests on patients known to be suffering from an infection, patients with uncontrolled thyroid dysfunction, or patients recovering from stress (e.g. surgery) as these alter insulin sensitivity.

2. Ensure that the child has had an adequate diet (minimum of 150 grams/day of carbohydrate) for at least 5 days before the test.

3. Fast the patient overnight (4 hours for infants) but avoid more prolonged fasting. Drinks of water only (no sweet drinks) are allowed during this period.

4. The child should not have had any breakfast on the day of the test.

Drug(s) Given

Rapilose OGTT solution (contains 75gm glucose in 300mL).

How Given

Calculate dose of Rapilose using dose calculator up to maximum of 75gm (300ml).

Give orally as quickly as possible without causing vomiting.

Timing of Administration

After baseline bloods taken.
Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Insert a reliable cannula</td>
</tr>
<tr>
<td></td>
<td>Take venous blood sample</td>
</tr>
<tr>
<td>0</td>
<td>Give Rapilose drink.</td>
</tr>
<tr>
<td></td>
<td>The drink should be fully consumed in 5 to 10 minutes</td>
</tr>
<tr>
<td>120</td>
<td>Take venous blood sample</td>
</tr>
<tr>
<td>30</td>
<td>If test is being carried out to investigate Insulin Resistance collect venous blood samples at these additional times</td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
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</table>

Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Standard Oral Glucose Tolerance Test</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>HbA1c</td>
</tr>
<tr>
<td>120</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Only if testing for insulin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>HbA1c (not for standard GTT)</td>
</tr>
<tr>
<td>30</td>
<td>Insulin</td>
</tr>
<tr>
<td>60</td>
<td>Insulin</td>
</tr>
<tr>
<td>90</td>
<td>Insulin</td>
</tr>
<tr>
<td>120</td>
<td>Insulin</td>
</tr>
</tbody>
</table>

Interpretation

WHO Criteria (from the Diagnosis and Classification of Diabetes Mellitus, Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003).

- A normal fasting plasma glucose is defined as less than 5.6 mmol/L.
- A normal glucose tolerance is defined by a 2-hour plasma glucose of less than 7.8 mmol/L.

Diagnosis of Diabetes

Diabetes should be diagnosed only when the 2-hour plasma glucose is 11.1 mmol/L or higher. In patients without symptoms diagnosis should not be based on a single glucose determination. At least one additional glucose test result with a value in the diabetic range is required, either fasting, random or 2 hours after a standard glucose load.
Impaired glucose tolerance
Defined by a fasting plasma glucose of less than 7.0 mmol/L and a 2 hour plasma glucose between 7.8 and 11.1 mmol/L.

Impaired fasting glycaemia
Defined by a fasting plasma glucose between 5.6 and 7.0 mmol/L and a 2 hour plasma glucose concentration of less than 7.8 mmol/L.

Insulin resistance
According to the recent consensus statement there are no clear cut offs to define insulin resistance in children and surrogate measures such as fasting insulin are not ideal.


Based on current screening criteria and methodology, there is no justification for screening children for insulin resistance related to obesity.

We recommend, in normal individuals:
- Fasting insulin concentration is:
  - less than 10 mU/L in children younger than 10 years of age.
  - Less than 20 mU/L in children older than 10 years.
- Peak insulin concentration is less than 100 mU/L.

In mild to moderate insulin resistance:
- Fasting insulin of 20 - 50 mU/L
- Peak insulin of 100 - 300 mU/L

In severe insulin resistance:
- Fasting insulin of greater than 50 mU/L
- peak insulin of greater than 300 mU/L
Luteinising Hormone Releasing Hormone (LHRH) Test (also known as GnRH Test)

Indications for the procedure

1. To assess the level of LH/FSH pituitary reserve.

2. To investigate pubertal disorders:
   a. precocious puberty
   b. premature breast development in girls (thelarche)
   c. delayed puberty

Preparations for the test

1. This test may be combined with Growth Hormone stimulation tests; increase the volumes of blood collected if combined with other tests.

2. Avoid HCG injections during the test as cross-reaction in LH analyses gives falsely elevated results.

3. Fasting is not required

4. Timing of the test is not important unless it is combined with the insulin hypoglycaemia test.

Drug(s) Given

Gonadorelin (LHRH, GnRH) 100 micrograms.

How Given

Intravenously as a slow bolus.

Timing of Administration

After baseline bloods taken.
### Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
</table>
| 0             | **Insert** a reliable cannula  
**Take** venous blood samples (basal samples)  
**Give** LHRH (GnRH, Gonadorelin) 100 micrograms i.v, over 2 minutes regardless of age. |
| 20 – 30       | **Take** venous blood sample |
| 60            | **Take** venous blood sample |

### Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>20 - 30</th>
<th>60</th>
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<tbody>
<tr>
<td>LH</td>
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<td>LH</td>
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<tr>
<td>FSH</td>
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<td>FSH</td>
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<tr>
<td>Girls - Oestradiol</td>
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<tr>
<td>Boys - Testosterone</td>
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</table>
Interpretation

The LHRH test can be difficult to interpret and results should be interpreted alongside clinical findings.

These include:

- Full pubertal staging
- Testicular volume in boy
- Ovarian ultrasound in girls

Puberty is a continuum and so is the response to the GnRH test.

Pre-pubertal response:

- LH peak less than 5U/L (or an LH increment less than 3-4U/L above basal).
- FSH peak greater than LH (or an FSH increment less than 2-3U/L above basal).

Peri-pubertal and pubertal response:

- Higher increments, especially if the LH response is dominant provides evidence of a pubertal pattern of gonadotrophin response.
- LH peak greater than 5U/L.
- LH peak greater than FSH peak.

Precocious puberty:

Gonadotrophin-independent precocious puberty:

- Spontaneous gonadotrophin secretion is suppressed by the autonomous sex steroid secretion
- Basal LH/FSH is low
- Response to LHRH is flat

Gonadotrophin-dependent precocious puberty:

- Basal LH/FSH levels are usually (but not always) elevated
- Response to LHRH is exaggerated.

Precocious puberty (treated):

- Suppressed basal LH/FSH
- Flat response to LHRH

Indicates adequate treatment with LHRH analogues. However, repeat LHRH test on treatment is not usually necessary.

Premature thelarche and thelarche variant:

- LH response is usually in the prepubertal range
- FSH response is predominant

Pubertal Delay and Pubertal failure:

Children with suspected hypogonadotrophic hypogonadism (HH):
• Complete lack of response supports the diagnosis.

However, a measurable but low response (in the pre-pubertal range) may occur both in HH and in constitutional delay of puberty and has limited predictive value.

Note that the LHRH test does not differentiate between HH and pubertal delay.

Primary gonadal failure:

• Basal LH and FSH are elevated
• LH and FSH response to GnRH is exaggerated.

References

HUMAN CHORIONIC GONADOTROPHIN (HCG) STIMULATION TEST

(From the Scottish DSD Network)

Introduction

Any child with an external masculinisation score (EMS, refer Figure 1) of below 11 requires further evaluation of the gonadal axis.

HCG stimulation of the testes may not be necessary in early infancy and prolonged hCG stimulation test is usually not necessary in infancy.

Under 6 months of age, a random measurement of testosterone, LH and FSH is all that is needed and avoid the hCG test.

For investigation of gonadal function, the standard hCG stimulation test performed over one week is sufficient in many cases but some cases may require prolonged hCG stimulation. The prolonged hCG stimulation test may be a useful means of investigating the endocrine gonadal axis after infancy when the testes are nascent. The test may also aid the descent of the undescended testes which are not completely impalpable.

The timing of the tests is important and the results may be influenced by age and the test may influence surgical and medical management.

If both gonads have never been palpable or detected, a karyotype should be sought before embarking on the hCG test, particularly in the apparent boy with premature virilization.

Figure 1
**Indications for the procedure**

To assess the ability of the testes to secrete testosterone.

To investigate causes of testicular disorders:

   a. Suspected anorchia
   b. Anatomical or developmental defects of the testes
   c. Enzyme defects of the testes
   d. Following torsion

**Preparations for the test**

1. If performing an LHRH test as well, the LHRH test should be performed before the hCG test.
2. If performing the hCG test first, the LHRH test should be performed at least 6 weeks after the hCG test.
3. Fasting is not required.
4. There are no contraindications to performing the test.
5. hCG may cause a degree of virilization (increase in testicular size, presence of erections) in boys with normal testes.

**Drug(s) Given**

Human Chorionic Gonadotrophin (hCG) 1500 units.

**How Given**

Intramuscularly.

**Timing of Administration**

After baseline bloods taken.
**Procedure and Samples Required**

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>&gt;Week 8</th>
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<td><strong>Thursday</strong></td>
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</tbody>
</table>

**Examine:**
- Stretched penile length
- Testes location
- Clinic review

**Administer:**
- hCG 1500iu, i.m.

**Measure:**
- Karyotype ³
- DNA ³
- AMH ³
- Urine steroid profile ³
- Androstenedione ²
- DHT ²
- DHEAS ²
- Testosterone ¹
- SHBG ¹

A short hCG test is usually done on day 1 and day 4. Samples can also be taken on day 5 in addition, if there are any issues with the day 4 sample.
Serum for Testosterone is very important; SHBG is much less important, particularly in infants.

These androgens are listed in order of priority with Androstenedione being most important.

These samples should preferably be collected on the first day but can be collected on any visit.

All children with a DSD should have an ultrasound scan of the renal tracts.

Interpretation

Interpretation of the results can be difficult.

The results should be discussed with a paediatric endocrinologist.

Follow-Up

Arrange endocrine clinic follow-up for 6 weeks after the completion of investigations.

Some children with a poor testosterone response to hCG may require further assessment of adrenal function.
Growth Hormone Stimulation Tests

Introduction

Growth hormone stimulation tests are often combined with other tests of pituitary function including the LHRH test and Synacthen test.

There are three stimulation tests commonly used for assessing the growth hormone axis:

- Insulin Hypoglycaemia Stress test.
- Arginine stimulation test
- Clonidine stimulation test

Other tests have been used for assessing the growth hormone axis. These are the Glucagon stimulation test and Exercise test. These tests are not recommended, due to problems with late hypoglycaemia and poor reproducibility of results.

‘Priming’ before the test

If the test is performed on peri-pubertal children (defined as having a bone age greater than 10 years, no signs of puberty in girls or testicular volume less than 8mL in boys), priming of the pituitary axis is often used. However, there is no consensus regarding whether priming is necessary.

When performing tests on the pituitary axis in these children, it is important to discuss with the local paediatric endocrinologist on the advisability of “priming” with the appropriate sex steroid.

Indications for procedure

To assess the ability of the anterior pituitary gland to secrete growth hormone (GH).

Used in the diagnosis of growth hormone deficiency.
**Insulin Hypoglycaemia Test**

**Introduction**

Commonly known as the Insulin Tolerance Test.

It is recognised as the “gold standard” for assessment of growth hormone deficiency, particularly as it also tests the hypothalamo-pituitary-adrenal axis.

This is a potentially high-risk test and **should only be carried out in a specialist centre where the test is being performed on a regular basis by experienced staff.**

Any form of stress results in the secretion of hypothalamic hormones, growth hormone releasing hormone (GHRH) and corticotrophin releasing hormone (CRH). These in turn stimulate the release of pituitary growth hormone (GH) and adrenocorticotrophic hormone (ACTH). ACTH release leads to cortisol secretion by the adrenal glands.

Insulin administration is used to produce stress in the form of hypoglycaemia, and hypothalamic pituitary adrenal (HPA) function is assessed by GH and cortisol responses to the hypoglycaemic stimulus.

This test is designed to produce symptomatic hypoglycaemia (pallor, sweating).

If symptoms are more severe (impaired or loss of consciousness) the child must be treated immediately (see below). Continuous observation for the symptoms of severe hypoglycaemia is essential throughout the test, and for one hour after its completion.

**Indications for procedure**

**Assessment of possible growth hormone deficiency in children with short stature, poor growth, survivors of childhood cancer treatment.**

**Assessment of anterior pituitary function.**

**Consistently abnormally low height velocity.**

**Contra-Indications for procedure**

Contraindicated in children with a history of epilepsy or cardiac arrhythmias. The test should be used with caution in young children, as symptoms of hypoglycaemia may be difficult to detect. Generally avoided in children under the age of 5 years.
Preparations for the test

1. The patient should be fasted overnight (no more than 4 hours for infants); drinks of water are allowed.

2. Weigh the patient and insert a cannula at least 30 minutes before taking the baseline samples. The patient should be resting throughout the test. Start the test between 0800h and 0900h.

3. Ensure that a glucose drink is available before the start of the test. This can be made up with four heaped teaspoons (equivalent to approximately 40g) dextrose powder dissolved in approximately half a glass of diluting juice. Alternatively, a standard glucose drink such as Lucozade 50 to 100mL can be used.

4. Ensure that glucose, and hydrocortisone are also available for intravenous injection if needed (see emergency treatment of severe hypoglycaemia, below).

5. Observe the child continuously during the test for symptoms of severe hypoglycaemia.

6. Check the glucose concentration in each blood sample collected using the ward blood glucose meter or more frequently if the child is developing hypoglycaemic symptoms.

7. If symptoms of severe hypoglycaemia develop they must be treated immediately. See below.

Drug(s) Given

Actrapid insulin 0.1 units per kg body weight.

How Given

Make up Actrapid insulin solution in 0.9% sodium chloride to give a solution containing 1 unit per ml.

Dose = 0.1 units per kg body weight.

Reduce the dose of insulin to be given to 0.05 units per kg in patients who might be unduly sensitive to insulin.

These include patients with a high clinical suspicion of hypopituitarism, those with severe malnutrition (e.g. due to anorexia nervosa) or those with a baseline blood glucose between 3.5 and 4.5 mmol/L.

Timing of Administration

Give after the two basal blood samples have been taken and the Capillary Blood Glucose is known.
Patient is most likely to become hypoglycaemia within first 30 mins of test and additional samples should be taken if and when there are signs/symptoms of hypoglycaemia.

Give a glucose drink when:

1. adequate hypoglycaemia has been established (a laboratory blood glucose of less than 2.2 mmol/L or a 50% reduction in the baseline glucose concentration)
2. if the child shows signs of hypoglycaemia (e.g. is sweaty and drowsy).
3. if there are more severe symptoms of hypoglycaemia (impaired or loss of consciousness), intravenous glucose may be required.

**NOTE:** ward meters frequently overestimate blood glucose concentrations.

**See emergency treatment guidelines below.**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>Insert a reliable cannula. Take venous blood sample. Measure capillary blood glucose (CBG) using ward meter.</td>
</tr>
<tr>
<td>0</td>
<td>Take venous blood sample. Measure CBG. If it is less than 3.5mmol/L in either of the two baseline samples, do NOT give insulin but continue to take blood samples (see below) and record whether child has symptoms (pale, sweating). If blood glucose, measured using the ward meter, is between 3.5 and 4.5mmol/L in either of the two baseline samples: Give half the dose of insulin and continue the test. Give all the dose of insulin if CBG is above 4.5mmol/L.</td>
</tr>
<tr>
<td>15</td>
<td>Take venous blood sample. Measure CBG.</td>
</tr>
<tr>
<td>30</td>
<td>Take venous blood sample. Measure CBG.</td>
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<tr>
<td>60</td>
<td>Take venous blood sample. Measure CBG.</td>
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<tr>
<td>90</td>
<td>Take venous blood sample. Measure CBG.</td>
</tr>
<tr>
<td>120</td>
<td>Take venous blood sample. Measure CBG.</td>
</tr>
</tbody>
</table>
## Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>-30</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
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<tbody>
<tr>
<td>Glucose</td>
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<td>T₄, TSH</td>
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<td>Oestradiol ¹</td>
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<td>Testosterone</td>
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</table>

¹ If LHRH is performed at the same time.
Emergency treatment of severe hypoglycaemia

If the child does not tolerate oral glucose or shows signs of severe hypoglycaemia (reduced conscious level) give intravenous glucose 200 mg per kg body weight (10% dextrose, 2mL per kg) over 3 minutes.

If the response is poor, give 100 mg hydrocortisone by intravenous injection.

Continue with a glucose infusion iv at 10 mg per kg per minute (6 mL/kg per hour of 10% dextrose).

Check blood glucose using the ward meter after 5 min and adjust the glucose infusion to maintain a blood glucose of 5-8 mmol/L and no higher.

If there is no improvement in conscious level after normal blood glucose is restored, an alternative explanation should be sought.

It is not necessary to discontinue the test and, if possible, continue blood sampling.

IMPORTANT

50% dextrose should NEVER be used in the resuscitation of a child with severe hypoglycaemia following an endocrine test.

After the test

- Once the test is completed, give a sweet drink and make sure the child has had something to eat.
- Keep the child under observation for at least 1 hour after the meal has been consumed.
- Keep the cannula in position until lunch has been eaten and the child has not vomited.
- Ensure that a blood glucose measured on the ward meter reads greater than 4 mmol/L before discharge.
- If there is any doubt about the child's wellbeing, keep him/her in the ward overnight for observation.

Interpretation of results

If Growth Hormone is greater than 6.7 microgram/l at any point this indicates there is a normal GH response and rules out growth hormone deficiency.

If Growth Hormone is less than 6.7 micrograms/l in the presence of adequate hypoglycaemia (blood glucose less than 2.2mmol/l or at least a 50% drop in plasma glucose, this indicates growth hormone deficiency.

Hypoglycaemia to this degree should also cause an increase in the plasma cortisol. Please check with local laboratories for reference ranges.
Arginine Stimulation Test

Indications for procedure

Short stature.
Consistent abnormally low growth velocity.
Assessment of possible growth hormone deficiency.
Where the ITT is not recommended or not suitable.

Preparations for the test
1. Patient to have water only for 8 hours prior to the test.
2. Arginine may cause nausea and some irritation at the infusion site.
3. In children with suspected hypopituitarism prolonged fasting may induce hypoglycaemia. Blood glucose should be checked by ward meter with each sample in these patients whenever a sample is taken.
4. This test can be combined with synacthen test to assess HPA axis, in addition to growth hormone deficiency.

Drug(s) Given
Arginine monochloride 0.5g/kg
Up to a maximum of 30g.

How Given
Dilute Arginine in equal quantities of 0.9% saline and infuse over 30 minutes

For example, dose 0.5gms per kilo
20kg child = 10gms of arginine= 20mls of arginine solution (5gms in 10mls)

Add 20mls of arginine solution to 20mls of 0.9% saline

Timing of Administration
Give after the first basal blood sample has been taken and the Capillary Blood Glucose is known.
### Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
</table>
| -30           | **Insert** a reliable cannula.  
**Take** venous blood sample.  
**Measure** capillary blood glucose (CBG) using ward meter.  
**Start** the arginine infusion and give over 30 minutes. |
| 0             | **Take** venous blood sample.  
**Measure** CBG. |
| 15            | **Take** venous blood sample.  
**Measure** CBG. |
| 30            | **Take** venous blood sample.  
**Measure** CBG. |
| 60            | **Take** venous blood sample.  
**Measure** CBG. |
| 90            | **Take** venous blood sample.  
**Measure** CBG. |
| 120           | **Take** venous blood sample.  
**Measure** CBG. |

### Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Glucose</th>
<th>GH</th>
<th>Cortisol</th>
<th>IGF₁</th>
<th>T₄, TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

₁ If synacthen test is performed at the same time.

### Interpretation

If the plasma GH concentration reaches 6.7 micrograms/L or more, further investigations are not indicated.

If the response is below this level, then an insulin hypoglycaemia test may be necessary.
Clonidine Stimulation Test

Indications for procedure

Investigation of suspected Growth Hormone (GH) deficiency in childhood (not in adults).

Clonidine is administered orally to provoke GH release.

Preparations for the test

1. Fast the patient overnight (4h for infants), and measure height and weight.
2. Calculate surface area from appropriate tables.
3. Start the test by 09:00h whenever possible.

Drug(s) Given

Clonidine 150 micrograms per m\(^2\) body surface area.
Round the dose up to the nearest 25 micrograms.

How Given

Given orally with a small sugar free drink.

Timing of Administration

Give after the two basal blood samples have been taken.
Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>Insert a reliable cannula. Take venous blood sample.</td>
</tr>
<tr>
<td>0</td>
<td>Take venous blood sample. Give oral clonidine dose.</td>
</tr>
<tr>
<td>15</td>
<td>Take venous blood sample.</td>
</tr>
<tr>
<td>30</td>
<td>Take venous blood sample.</td>
</tr>
<tr>
<td>60</td>
<td>Take venous blood sample.</td>
</tr>
<tr>
<td>90</td>
<td>Take venous blood sample.</td>
</tr>
<tr>
<td>120</td>
<td>Take venous blood sample.</td>
</tr>
<tr>
<td>150</td>
<td>Take venous blood sample.</td>
</tr>
</tbody>
</table>

Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>GH</th>
<th>IGF₁</th>
<th>T₄, TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After the test

- Once the test is completed, make sure the child has had something to eat and drink.
- Side effects of Clonidine (hypotension and drowsiness) may persist for several hours after the test.
- Keep the patient lying down for at least an hour after the test.
- Check pulse and blood pressure half-hourly and also before allowing the child to get up.
- Careful observation of the patient is necessary until late afternoon.

Interpretation

If the plasma GH concentration reaches 6.7 micrograms/L or more, further investigations are not necessary.
If the response is below this level, then an insulin hypoglycaemia test may be necessary.
Standard Short Synacthen Test

Indications for procedure

Assessment of adrenocortical function.

The synthetic polypeptide Synacthen (Tetracosactrin BP) has a structure identical to the N-terminal 24 amino acids of Adrenocorticotropic Hormone (ACTH).

It has a short duration of action and permits a rapid and convenient screening test for the assessment of adrenocortical function by measuring cortisol response.

Measurement of additional adrenal steroids during the test (at the same time as the samples for cortisol - see below) may also be used to assess the steroid biosynthetic pathway.

Plasma 17-hydroxyprogesterone (17-OHP) measurements may assist in the diagnosis of non-salt-losing congenital adrenal hyperplasia.

If a defect in steroid biosynthesis is suspected, a random urine specimen for a full steroid profile taken before the Synacthen test may be helpful.

Preparations for the test

1. Prednisolone and hydrocortisone both interfere with the measurement of cortisol.
2. If the patient is already on hydrocortisone ask the patient to omit their doses the evening before and on the morning of the test.
3. The patient should continue taking the hydrocortisone immediately after the test whilst waiting for results.
4. The Synacthen test should not be performed if the patient has been on Prednisolone within the last 2 weeks.
5. The patient does not need to fast.
6. A cannula should be inserted at least 30 minutes before taking the baseline sample.

Drug(s) Given

Synacthen (Tetracosactrin BP) 250 micrograms.

For neonates and infants less than 7kg, give 36 micrograms/kg (rounded to the nearest 25 micrograms)

How Given

Intravenous bolus.

Timing of Administration

Give after basal sample has been taken.
Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Insert a reliable cannula, Take venous blood sample, Give Synacthen</td>
</tr>
<tr>
<td>30</td>
<td>Take venous blood sample</td>
</tr>
<tr>
<td>60</td>
<td>Take venous blood sample</td>
</tr>
</tbody>
</table>

Samples Required

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Cortisol</td>
</tr>
<tr>
<td>30</td>
<td>Cortisol</td>
</tr>
<tr>
<td>60</td>
<td>Cortisol</td>
</tr>
</tbody>
</table>

Interpretation

A normal Cortisol response to synacthen at 60 min is greater than 430 nmol/L (Abbott Assay).

The exact values of cortisol cutoffs will be affected by the laboratory method used. Please consult your local laboratory.

Interpretation of plasma 17-OHP depends on age and clinical presentation.

Both adults and children usually have baseline 17-hydroxyprogesterone concentrations less than 6 nmol/l.

In late onset congenital adrenal hyperplasia, the baseline 17-hydroxyprogesterone may be either normal or high, but there is an exaggerated response (>30nmol/l) to Synacthen stimulation.
**Water Deprivation Test**

**Indications for procedure**

*Used when the diagnosis of Diabetes Insipidus (DI) is in doubt.*

As most cases of DI can be confirmed or excluded on history and baseline investigations, this test is generally not recommended.

**This test can be potentially dangerous** and is very distressing to the patient.

We suggest patients should be discussed with a paediatric endocrinologist before performing the test.

It may be more appropriate for the test to be carried out in a tertiary centre.

**For this reason, a protocol for the water deprivation test is not part of this handbook**

**PSYCHOGENIC POLYDIPSIA**

The vast majority of children who appear to be drinking excessive amounts of fluid have “psychogenic polydipsia” (habitual drinking) and are usually able to concentrate their urine appropriately.

Many have become habitual juice drinkers and will reduce their intake if only water is offered.

Assessment of these children should be made after allowing access to water but restricting juice and other flavoured fluids.

(Children with DI will continue to drink large amounts).

**Procedure for assessing psychogenic polydypsia**

1. Initially check an early morning urine for osmaolarity following a period of only drinking water.

2. Unusual features more likely to be seen in Diabetes Insipidus are:
   a. drinking unusual fluids such as bath water, pets water.
   b. drinking through the night.
   c. new onset enuresis.

**Samples Required**

Urine osmolality is all that is required for initial assessment.
Interpretation

1. A random urine osmolality of more than 800 mosm/l will exclude Diabetes Insipidus.

2. If the random urine osmolality is less than 300 mosm/L, check an early morning fasting urine osmolality.

3. Child should be allowed water only to drink the day before (no juice etc.).

   If DI is still considered, please discuss with your tertiary centre.
Dexamethasone Suppression Tests

Introduction

There are several forms of the dexamethasone suppression test:

1. Overnight dexamethasone suppression test
2. Low dose dexamethasone suppression test
3. High dose dexamethasone suppression test

The overnight dexamethasone suppression test is used as an initial screening test. If there is a failure of suppression, a prolonged dexamethasone test may be required. This should be discussed with your local tertiary endocrinologist.

In this case a prolonged (low or high dose) Dexamethasone suppression test may help confirm Cushing’s syndrome and identify the cause.
Overnight Dexamethasone Suppression Test

Indications for procedure

To identify Cushing’s Syndrome.

The Overnight Dexamethasone Suppression Test is used as an initial screening test.

Preparations for the test

No specific preparation required.

Ensure the patient is not taking steroids in any form.

Drug(s) Given

Dexamethasone 10 micrograms per kg body weight up to a maximum of 1 mg.

Available as scored 500 microgram tablets.

Round the dose to the dearest 250 micrograms (i.e. half a tablet).

How Given

Orally in tablet form.

Timing of Administration

Give the dose of dexamethasone between 23:00 and midnight.

Procedure

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>23:00 to 00:00</td>
<td>Give dexamethasone orally.</td>
</tr>
<tr>
<td>09:00 next day</td>
<td>Take blood sample.</td>
</tr>
</tbody>
</table>

Samples Required

<table>
<thead>
<tr>
<th>Time</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td></td>
</tr>
</tbody>
</table>
Interpretation

In normal subjects, the morning plasma cortisol concentration is suppressed to less than 50 nmol/l.

In patients with Cushing’s syndrome, such marked suppression is not observed.

Patients taking hepatic enzyme-inducing drugs (e.g. phenytoin, phenobarbitone) may have false negative results.
Low Dose Dexamethasone Suppression Test

Indications for procedure
Diagnosis of Cushing's syndrome

Preparations for the test
A 24-hour urine collection for urinary free cortisol and urinary steroid profiles should be taken before dexamethasone is given.

Drug(s) Given
Dexamethasone 0.5 mg every 6 hours for two days total of eight doses
In younger children give 20 micrograms/kg/dose every 6 hours.
Maximum single dose is 0.5 mg.

How Given
Orally in tablet form.

Timing of Administration
Start oral dexamethasone at on the morning following completion of urine collection (day two).

Procedure

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 @ 09:00</td>
<td>Take venous blood sample</td>
</tr>
<tr>
<td></td>
<td>Start urine collection.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 @ midnight</td>
<td>Take venous sample.</td>
</tr>
<tr>
<td>Day 2 @ 06:00, 12:00, 18:00, 00:00</td>
<td>Give dexamethasone 6 hourly.</td>
</tr>
<tr>
<td>Day 3 @ 06:00, 12:00, 18:00, 00:00</td>
<td>Give dexamethasone 6 hourly.</td>
</tr>
<tr>
<td>Day 4 @ 06:00</td>
<td>Take venous sample.</td>
</tr>
<tr>
<td></td>
<td>Start urine collection.</td>
</tr>
</tbody>
</table>
Samples Required

<table>
<thead>
<tr>
<th>Time</th>
<th>Cortisol</th>
<th>ACTH</th>
<th>Urine steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 @ 09:00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 @ midnight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4 @ 06:00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation

In normal circumstances, Cortisol concentrations should suppress to less than 50nmol/L.

In patients with Cushing’s syndrome from whatever cause, there is loss of the normal negative feedback control by circulating glucocorticoids on ACTH release.

These patients will exhibit detectable plasma ACTH and cortisol concentrations after dexamethasone administration.

In patients who fail to show cortisol suppression, a pre-test ACTH level of less than 5ng/L is highly suggestive of an adrenal cause of Cushing’s syndrome.
High dose Dexamethasone Suppression Test

Indications for procedure
To differentiate between pituitary-dependent and ectopic causes of Cushing’s syndrome.

Preparations for the test
No special preparations are required.

Drug(s) Given
Dexamethasone tablets available as 500 microgram tablets.
Give 2mg every 6 hours for a total of eight doses.
In younger children give 80 micrograms/kg/dose every 6 hours for a total of eight doses.
Maximum single dose is 2mg.

How Given
Orally on day two of the test.

Timing of Administration
After basal urine and blood samples have been taken.

Procedure

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 @ 09:00</td>
<td><strong>Take</strong> venous blood sample. <strong>Start</strong> 24 hour urine collection.</td>
</tr>
<tr>
<td>Day 1 @ midnight</td>
<td><strong>Take</strong> venous blood sample.</td>
</tr>
<tr>
<td>Day 2 @ 06:00, 12:00, 18:00, 00:00</td>
<td><strong>Take</strong> oral dexamethasone 6 hourly</td>
</tr>
<tr>
<td>Day 3 @ 06:00, 12:00, 18:00, 00:00</td>
<td><strong>Take</strong> oral dexamethasone 6 hourly</td>
</tr>
<tr>
<td>Day 4 @ 06:00</td>
<td><strong>Take</strong> venous blood sample. <strong>Start</strong> 24 hour urine collection.</td>
</tr>
</tbody>
</table>
Samples Required

<table>
<thead>
<tr>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 @ 09:00</td>
<td>Take venous blood sample</td>
</tr>
<tr>
<td></td>
<td>Start urine collection.</td>
</tr>
<tr>
<td>Day 1 @ midnight</td>
<td>Take venous sample.</td>
</tr>
<tr>
<td>Day 2 @ 06:00, 12:00, 18:00, 00:00</td>
<td>Give dexamethasone 6 hourly.</td>
</tr>
<tr>
<td>Day 3 @ 06:00, 12:00, 18:00, 00:00</td>
<td>Give dexamethasone 6 hourly.</td>
</tr>
<tr>
<td>Day 4 @ 06:00</td>
<td>Take venous sample.</td>
</tr>
<tr>
<td></td>
<td>Start urine collection.</td>
</tr>
</tbody>
</table>

Interpretation

In Pituitary-dependent hypercortisolism (Cushing’s disease), the plasma cortisol concentration usually suppresses to at least 50% of basal values.

In approximately 10% of patients with Cushing’s disease there is a failure to suppress cortisol.

Approximately 10% of patients with ectopic ACTH secretion will suppress.

It is important to discuss the use of dexamethasone suppression and the interpretation of the results with a paediatric endocrinologist.