ICE Test Name: 3 Day HCG Stimulation test (request base and Day 3)

**Principle**

hCG is a polypeptide hormone and shares a common subunit with LH. It stimulates testicular Leydig cells to secrete androgens via the LH receptors. Children aged 6 months to 8 years frequently have undetectable basal gonadal steroids in plasma and gonadal function can only be assessed by Leydig cell stimulation using hCG.

**Indication**
- To detect functioning testicular tissue in the investigation of male hypogonadism, ambiguous genitalia, micropenis, delayed puberty and/or undescended testes. The test should be performed even if the gonads are impalpable (and the karyotype is XY or XY mosaic).
- To define enzyme blocks in testosterone biosynthesis.

**Precautions**
- In boys with normal testes there may be some virilisation (increase in testicular size, erections).
- The test should not be performed before 2 weeks of age.
- If a GnRH test is planned, this should be carried out before the HCG test (or > 6 weeks after) as HCG has a long half-life.

**Side Effects**
- Headaches and/or tiredness are reported side effects.

**Preparation**
- None required.

**Protocol**

3 Day Protocol:
1. **Day 1** - Between 8.00 a.m. and 9.00 a.m. collect baseline blood samples for testosterone (also androstenedione and dihydrotestosterone if a steroid biosynthetic defect is suspected).
2. Immediately following collection of baseline blood samples, give hCG i.m. as follows:
   - 500 IU if weight < 5kg
   - 1000 IU if weight 5 - 10kg
   - 1500 IU if weight 10 - 15kg
   - 3000 IU if weight above 15kg
3. **Day 4** - Repeat blood sample 72 hours after hCG injection for testosterone, dihydrotestosterone and androstenedione.

If the results of the 3 day test are equivocal then consider performing the 3 week hCG stimulation test.

**Samples**

- **Testosterone, DHT & Androstenedione**
  - 4 mL clotted blood (white top)
- **Urinary Steroid Profiling**
  - 24h urine in a plain bottle if indicated

**Record actual sample collection times on the printed barcodes.**

**Interpretation**

The normal testosterone response depends on the age of the patient. In infancy, a normal testosterone increment after hCG may vary from 2-fold to 10- or even 20-fold. During childhood, the increment is between 5- and 10-fold. During puberty, as the basal concentration is higher, the increment is less, i.e. 2- to 3-fold. In the absence of testes, no response to testosterone occurs.

An absent response with an exaggerated LH/FSH response to LHRH stimulation indicates primary gonadal failure or anorchia. If there is a defect in testosterone biosynthesis, there will be an increase in precursor steroid secretion following HCG stimulation.
In normal male children (6 months – puberty) the T/DHT ratio is <20 before HCG stimulation and <27 after HCG stimulation. In 5α-reductase deficiency the T/DHT ratio is <20 before HCG stimulation but >27 after stimulation. There are reported errors in the interpretation of the hCG stimulation test in boys ~8yrs of age with increased Testosterone:DHT in the 5α-reductase range.

In 17β-hydroxysteroid dehydrogenase deficiency the androstenedione:testosterone ratio is >20 post hCG.

Samples are sent to Leeds General Infirmary for analysis. Leeds have recently changed their interpretation cut offs following a change to LCMSMS measurement rather than previously validated RIA results as follows:

<table>
<thead>
<tr>
<th></th>
<th>Testosterone (nmol/L)</th>
<th>DHT (nmol/L)</th>
<th>T/DHT ratio after hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal male adults</td>
<td>8 - 27</td>
<td>&lt; 2.9</td>
<td>&lt; 12</td>
</tr>
<tr>
<td>Normal children (6 months – puberty)</td>
<td>&lt; 0.9</td>
<td>&lt; 0.1</td>
<td>&lt; 12</td>
</tr>
<tr>
<td>5α-reductase deficiency (6 months – puberty)</td>
<td></td>
<td></td>
<td>&gt; 12</td>
</tr>
</tbody>
</table>

References