PAEDIATRIC INVESTIGATIONS: GUIDELINES FOR SPECIALIST BIOCHEMICAL TESTS

Version:	2.0
Ratified by:	Clinical Biochemistry Senior Staff Meeting
Date ratified:	
Name of originator/author:	Miss Elizabeth Hall
Director responsible for implementation:	Dr E Lamb
Date issued:	January 2020
Review date:	January 2022
Target audience:	Clinical staff (medical, nursing and scientific), Trust wide

Version Control Schedule

Version	Date	Author	Status	Comment
1.0	May 2016	Miss Elizabeth Hall		
2.0	Jan 2020	Miss Elizabeth Hall		Amended 17OHP cut-offs during short synacthen test in line with new provider of assay

Contents

1 Policy Summary

This policy gives guidance on how to perform a range of specialist biochemical investigations on paediatric patients.

2 Introduction

Some conditions, mostly of an endocrine nature, require specialist biochemical investigation utilising dynamic function tests to confirm or exclude a diagnosis. Often such investigations are complicated, subject to variation in how they are performed and the interpretation challenging. The aim of this document is to provide a range of specialist biochemical investigations for children that can be used throughout the Trust providing best clinical practice. Before any investigation is undertaken the clinical indication should be discussed and agreed with a consultant paediatrician or the Duty Biochemist (ext 723-6287).

3 Purpose and Scope

This policy outlines a number of approved specialist biochemical investigations in paediatric patients. It may only be used for patients within the Trust under consultant paediatrician guidance.

4 Definitions

Dynamic function test – a series of samples are collected at defined time points before and after an intervention to assess the response to that intervention.

Sample tubes – all estimates of the number of blood sample tubes required assumes that full 2 mL tubes are collected. The sample top colours assume that standard Greiner evacuated tubes are used.

Red top sample tubes – serum tubes with clot activator, no gel

Gold top sample tubes – serum separator tubes with gel

Purple top sample tubes – contain potassium EDTA

Grey top sample tubes – contain sodium fluoride and potassium oxalate

Green top tubes – contain lithium heparin

IGF-1 – insulin-like growth factor 1

5 Duties

All staff involved in performing a specialist biochemical investigation, whether clinical or laboratory, must adhere to this policy.

Clinical staff must ensure that there are sufficient clinical details on the request form to justify the request. This should include the name of the substance to be administered and the indication for the test.

6 Tests that should only be performed at a tertiary referral centre

There are no protocols in this document for the following tests:

Controlled prolonged fast for investigating hypoglycaemia

Insulin stress test – the glucagon tolerance test is preferred

TRH test

Water deprivation test

These tests should only be performed at a tertiary referral centre and after discussion with one of the visiting consultant paediatricians.

7 Oral glucose tolerance test – protocol for paediatrics

Indications for Test

An oral glucose tolerance test is performed to exclude/confirm the diagnosis of diabetes mellitus. In patients with characteristic symptoms of diabetes (e.g. weight loss, thirst, polyuria) or metabolic decompensation (e.g. ketoacidosis), a single random glucose concentration often confirms the diagnosis. For individuals presenting with subtler symptoms, measurement of fasting plasma glucose concentration is essential. If the fasting glucose concentration is equivocal, an oral glucose tolerance test must then be performed, to assess the ability of the individual to handle a glucose load.

Before subjecting a patient to an oral glucose tolerance test (GTT), ensure that there has been an appropriate diagnostic work-up (see WHO guidelines). During the GTT, blood samples are collected for measurement of plasma glucose before (fasting) and 2 hours after administration of an oral glucose load. Polycal liquid (previously called Fortical) is used as the glucose load. Adherence to the following instructions will ensure the test is conducted in accordance with the recommendations of the World Health Organisation. If you require any further information or clarification please contact the duty biochemist on telephone number 01233 616287 (ext 723-6287).

Contraindications

An oral glucose tolerance test must not be performed if the fasting capillary (finger prick) or venous blood glucose concentration is greater than 10 mmol/L.

Requirements

- Two timed blood (2 mL) samples collected into fluoride oxalate (grey top) sample tubes (see below for patient preparation)
- It is essential that the blood samples are processed by the laboratory: results obtained using blood glucose meters are of no value in establishing or refuting the diagnosis of diabetes mellitus.
- Polycal liquid containing 61.4 g maltodextrin per 100 mL.
- Measuring cylinder. These can be obtained from the Pathology Laboratory on request.

Patient Preparation

The patient must have fasted for at least 8 hours, and no more than 14 hours (water is permitted). The patient must have been following their normal carbohydrate diet for three days preceding the test.

Procedure

- Confirm the patient's details and that he/she has fasted on the morning of the test. If the patient has eaten on the morning of the test, the test must be abandoned and a repeat appointment arranged.
- Explain the nature of the procedure to the patient. Two blood samples will be collected, 2 hours apart, before and after the Polycal drink.
- Using a glucose meter, determine the patient's fasting blood glucose concentration with a capillary blood sample obtained by finger prick.
- The result must be between 4 and 10 mmol/L. If outside this range, the Paediatric Consultant must be informed. We do not recommend continuing with the test. Instead, take a venous sample of blood for a fasting glucose concentration and send it to the laboratory to confirm the result obtained on the glucose meter.
- Providing the glucose meter result is between 4 and 10 mmol/L, proceed with the test. Blood (2 mL) must be collected into a fluoride oxalate sample tube tube. Record full patient details on the collection bottle including the test time (i.e. time zero/fasting). Record the glucose meter result on the laboratory request form.
- The Polycal must then be administered. **DO NOT GIVE THE WHOLE BOTTLE OF POLYCAL**.
- The dose of Polycal must be adjusted for weight of the child. The appropriate dose is 1.75 g glucose/kg body weight (to a maximum adult load of 75 g anhydrous glucose). This is equivalent to 2.64 mL Polycal/kg body weight (to a maximum of 113 mL Polycal, equivalent to a 75 g glucose load).

  Dilute the measured Polycal with an equal volume of water. This must be drunk over the course of 5 minutes or less. Immediately give a further 50 mL water.
- Note the time the Polycal was given on the request form.
- The patient must sit quietly during the test and not leave the department or eat or drink anything. After **exactly** 2 hours, collect a further blood (2 mL) sample and record full patient details on the collection bottle including the actual time and time post glucose load (i.e. time 2 hours).
- The test is complete. The patient may eat and drink normally again and is free to leave. Send the blood samples to the Pathology Laboratory for analysis as soon as possible.

Interpretation

	Glucose concentration (mmol/L)
Diabetes mellitus:	
Fasting ***or***	≥7.0
2 h post glucose load	≥11.1
or both	
Impaired glucose tolerance (IGT):	
Fasting (if measured) ***and***	<7.0
2 h post glucose load	≥7.8 and <11.0
Impaired fasting glycaemia (IFG):	
Fasting	≥6.1 and ≤6.9
and *(in measured)* 2 h post glucose	<7.8

Laboratory results will be issued with an interpretative comment. The East Kent Hospitals University NHS Foundation Trust diagnostic algorithm for diabetes mellitus can be found on Sharepoint. If you require any further advice with respect to the interpretation of the test results, please contact the duty biochemist on 01233 616287 (ext 723-6287).

References

1. World Health Organisation. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Geneva, World Health Organisation, 2006.

2. Colley CM, Larner JR. The use of Fortical in glucose tolerance tests. Ann Clin Biochem 1990; 27:496-98.

3. Smith J and Natrass M. Diabetes and laboratory medicine. ACB Venture publications 2004.

8 Extended oral glucose tolerance test for insulin resistance in SGA/IUGR patients – protocol for paediatrics

Indications for Test

Assessment of insulin resistance in small for gestational age (SGA) or interuterine growth retardation (IUGR) children before or during growth hormone (GH) treatment.

Patient Preparation

The child should be fasted from midnight with only water to drink.

Requirements

- Fluoride oxalate (grey top) sample tubes for glucose measurement
- Plain (red top) sample tubes for insulin measurement
- Polycal liquid containing 61.4 g maltodextrin per 100 mL.
- Measuring cylinder. These can be obtained from the Pathology Laboratory on request.

Procedure

- The child should attend the ward between 08:45–09:00
- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour.
- Cannulate the patient
- Take samples (time 0).
- **DO NOT GIVE THE WHOLE BOTTLE OF POLYCAL**. The dose of Polycal must be adjusted for weight of the child. The appropriate dose is 1.75 g glucose/kg body weight (to a maximum adult load of 75 g anhydrous glucose). This is equivalent to 2.64 mL Polycal/kg body weight (to a maximum of 113 mL Polycal, equivalent to a 75 g glucose load).
  Dilute the measured Polycal with an equal volume of water. This must be drunk over the course of 5 minutes or less. Immediately give a further 50 mL water.
- Note the time the Polycal was given on the request form.
- Take remaining samples following the table below. Ensure each sample and form is clearly marked with the actual collection time as well as 0, 30 minutes etc.

Time (mins)	Glucose (Grey)	Insulin (Red)
0	+	+
30	+	+
60	+	+
90	+	+
120	+	+

- A bedside check using a glucose meter on fingerprick blood samples will give a provisional indication of the glucose concentration.
- Additional bloods may be collected at any time during the test:
  Liver function tests (gold top)
  Lipid risk profile: Cholesterol/TG/HDL:LDL ratio (gold top)
- The patient should not exercise during the test. They should have nothing else to eat or drink during the 2 hours of the test, as even water may influence the rate of glucose absorption.

Interpretation

The baseline insulin sample will be analysed for all patients. The other insulin samples will be stored for one month and will be available for analysis if the glucose results demonstrate impaired glucose tolerance. Testing of stored insulin samples must be by specific request from Dr Buchanan and arranged with the Duty Biochemist (x723-6287, DDI 01233 616287)

References

Dr Buchanan, personal communication 1-6-15

9 Extended oral glucose tolerance test for diagnosing growth hormone excess – protocol for paediatrics

Indications for Test

This test is used for the diagnosis of acromegaly and pituitary gigantism.

Patient Preparation

The child should be fasted from midnight, with only water to drink.

Requirements

- Fluoride oxalate (grey top) sample tubes for glucose measurement
- Plain (red top) sample tubes for growth hormone and IGF1 measurement
- EDTA (purple top) sample tube for HbA1c measurement
- Polycal liquid containing 61.4 g maltodextrin per 100 mL.
- Measuring cylinder. These can be obtained from the Pathology Laboratory on request.

Procedure

- The child should attend the ward between 08:45–09:00.
- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour.
- Cannulate the patient and take baseline samples (-30).
- Take samples (time 0).
- **DO NOT GIVE THE WHOLE BOTTLE OF POLYCAL**. The dose of Polycal must be adjusted for weight of the child. The appropriate dose is 1.75 g glucose/kg body weight (to a maximum adult load of 75 g anhydrous glucose). This is equivalent to 2.64 mL Polycal/kg body weight (to a maximum of 113 mL Polycal, equivalent to a 75 g glucose load).
  Dilute the measured Polycal with an equal volume of water. This must be drunk over the course of 5 minutes or less. Immediately give a further 50 mL water.
- Note the time the Polycal was given on the request form.
- Take remaining samples following the table below.

Analyte	**Time (Minutes)**					
	-30	0	30	60	90	120
Glucose	+	+	+	+	+	+
Growth hormone	+	+	+	+	+	+
IGF-1	+					
Grey top samples	1	1	1	1	1	1
Serum samples	2	1	1	1	1	1

- Ensure each sample and form is clearly marked with the actual collection time as well as 0, 30, 60 minutes etc.

- A bedside check using a glucose meter on fingerprick blood samples will give a provisional indication of the glucose concentration.
- The patient should not exercise during the test. They should have nothing else to eat or drink during the 2 hours of the test, as even water may influence the rate of glucose absorption.

Interpretation

A normal response would be suppression of serum growth hormone to undetectable concentrations at any time point during the test.

References

1. Barth et al. Biochemical Investigations in Laboratory Medicine http://www.pathology.leedsth.nhs.uk/dnn_bilm/Home.aspx (accessed 18-11-15)

10 Short Synacthen test for adrenal hypofunction – protocol for paediatrics

Indications for Test

A short Synacthen test is performed for the diagnosis/exclusion of adrenal hypofunction (including Addisonian crisis). Indications include hyponatraemia, hypotension, hypoglycaemia, uraemia and/or an equivocal 09:00 cortisol concentration.

Contraindications

Hydrocortisone and fludrocortisone interfere with this test. If safe, steroid therapy should be discontinued the evening prior to performing the short Synacthen test. Steroid therapy can be recommenced immediately after the short Synacthen test has been performed. The short Synacthen test gives unreliable results in the two weeks following pituitary surgery.

Patient Preparation

There are no dietary restrictions for this test. Patients should not be receiving steroid therapy. Hydrocortisone must be stopped for 12 hours before the test.
The test should, ideally, be performed at 09:00.

Side Effects

There are rare reports of hypersensitivity to Synacthen.
Caution is required in patients with a history of atopic allergy such as asthma, eczema and hayfever.

Requirements

- Plain (red top) sample tubes for cortisol measurement
- EDTA (purple top) sample tube for ACTH measurement
- Tetracosactide (synacthen) ampoule, 250 µg/mL

Procedure

- All cortisol samples should be collected before commencing steroid therapy.
- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour.
- Ideally perform test at 09:00.
- Take blood samples for serum cortisol and adrenocorticotrophin hormone (ACTH) at 09:00 (time 0). Please write the **actual time** on both the sample and form.
  The ACTH sample must reach the laboratory within 10 minutes to allow processing within 15 minutes. Samples must be delivered by hand and must not be sent by pod.
- Synacthen is administered through the cannula. It may be diluted in normal saline.

- For children the following Synacthen doses are recommended:

< 6 months age	62.5 µg	equivalent to 0.25 mL
6-24 months age	125 µg	equivalent to 0.5 mL
> 2 years age	250 µg	equivalent to 1.0 mL

- Take a second blood sample for serum cortisol **exactly 30 minutes post** Synacthen injection. Please write the **actual time** on both the sample and form.
- Send **both cortisol samples together** to the laboratory with the request form for a short Synacthen test.

Interpretation

Assuming the patient is not on steroids, a serum cortisol concentration 30 minutes post Synacthen administration ≥480 nmol/L is a normal response and excludes primary adrenal hypofunction. A normal response does not exclude secondary (pituitary) adrenal hypofunction.

An equivocal response, a serum cortisol concentration 30 minutes post Synacthen administration between 450 and 479 nmol/L, may require further assessment of adrenal reserve and a depot (1 mg) Synacthen test to be performed.

If an inadequate response is obtained, a serum cortisol concentration 30 minutes post Synacthen <450 nmol/L, or there is clinical suspicion of secondary adrenal hypofunction the ACTH sample will be sent for analysis.

References

1. Barth et al. Biochemical Investigations in Laboratory Medicine http://www.pathology.leedsth.nhs.uk/dnn_bilm/Home.aspx (accessed 18-11-15)
2. Clark PM, Neylon I, Raggatt PR et al. Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. Clin Endocrinol 1998;**49**:287-92
3. Wallace I, Cunningham S and Lindsay J. The diagnosis and investigation of adrenal insufficiency in adults. Ann Clin Biochem 2009;**46**:351-67
4. Chatha KK, Middle JG & Kilpatrick ES. National UK audit of the short Synacthen test. Ann Clin Biochem 2010;**47**:158-64
5. Howlett, TA. An assessment of optimal hydrocortisone replacement therapy. Clin Endo 1997; **46**, 263-268
6. Carter JL, Anslow T. Comparison of Bayer ADVIA Centaur Cortisol and Abbott ARCHITECT Cortisol Immunoassays. S:\Path\Staff\ClinBio\Method evaluations\2008 \Cortisol method evaluation 2008.doc

11 Short Synacthen test for diagnosing congenital adrenal hyperplasia - protocol for paediatrics

Indications for Test

This is performed for the investigation of congenital adrenal hyperplasia (CAH) in children. In patients with a deficiency in the steroid synthesis pathway cortisol may not be adequately secreted. However, there is excessive secretion of the precursor steroids prior to the defective enzyme. The commonest form of CAH is due to the deficiency of 21-hydroxylase and in these subjects increased secretion of 17-OH progesterone can be detected.

Contraindications

Hydrocortisone and fludrocortisone interfere with this test. If safe, steroid therapy should be discontinued the evening prior to performing the short Synacthen test. Steroid therapy can be recommenced immediately after the short Synacthen test has been performed. The short Synacthen test gives unreliable results in the two weeks following pituitary surgery. Tetracosactide (Synacthen) is contraindicated in patients with a history of atopic allergy such as asthma, eczema and hayfever.

Patient Preparation

There are no dietary restrictions for this test. Patients should not be receiving steroid therapy. The test should, ideally, be performed at 09:00.

Side Effects

There are rare reports of hypersensitivity to Synacthen.

Requirements

- Plain (red top) sample tubes for cortisol and 17-OH progesterone measurement
- Tetracosactide (Synacthen) ampoule, 250 µg/mL

Procedure

- All cortisol samples should be collected **before** commencement of steroid therapy.
- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour.
- Ideally perform test at 09:00.
- Take samples (time 0).
- Synacthen is administered through the cannula. It may be diluted in normal saline.

- For children the following Synacthen doses are recommended:

< 6 months age	62.5 µg	equivalent to 0.25 mL
6-24 months age	125 µg	equivalent to 0.5 mL
> 2 years age	250 µg	equivalent to 1.0 mL

- Continue the sampling using the table below.
  Ensure each sample and form is clearly marked with the actual collection time as well as 0, 30 minutes etc.

Time (mins)	**0**	**30**
Cortisol	+	+
17-OH Progesterone	+	+
Gold top samples	2	2

Interpretation

A normal cortisol response is indicated by a rise in cortisol concentration to > 480 nmol/L at 30 minutes post Synacthen.

17-OH progesterone reference ranges (nmol/L)

	basal	30 minutes post Synacthen
Normal response	<8.9	<8.9
CYP21-defect CAH	≥8.9	≥8.9

References

1. 17-OH progesterone reference ranges from University Hospital South Manchester (personal communication)

12 Glucagon test to assess HPA axis – protocol for paediatrics

Indications for Test

The glucagon test assesses the hypothalamic-pituitary-adrenal (HPA) axis; glucagon stimulates the release of growth hormone (GH) and ACTH by a hypothalamic mechanism and therefore indirectly stimulates cortisol. This test is useful in young children, <2 years of age, in whom a reliable IV line may be difficult to achieve and in any child where insulin induced hypoglycaemia is contraindicated e.g. a history of convulsions, hypoglycaemia or diabetes mellitus. The insulin stress test should only be performed at a tertiary referral centre.

Contraindications

This test should not be performed in subjects with hypothyroidism or adrenal failure. This test is unreliable in patients with diabetes mellitus.

Requirements

Patients must be seen by a paediatrician with a special interest in endocrinology or by a paediatric endocrinologist before undergoing this test.

- Glucagon
- Liquid drink (squash/milk) and food (toast) should be available
- Strong oral glucose solution
- Intravenous glucose and hydrocortisone must be available at the bedside.
- Fluoride oxalate (grey top) sample tube tubes for glucose measurement
- Serum (gold top) sample tube tubes for growth hormone and cortisol measurement

Side Effects

Nausea and vomiting may occur.

Hypoglycaemia can occur in children after administration of glucagon.

Patient Preparation

The patient must fast from midnight the night before the test, with only water to drink, and no breakfast on the morning of the test.

Priming with sex steroids is recommended in prepubertal children who are over 10 years of age (either chronological or bone age) especially if the LHRH test is being performed at the same time. Prescribe stilboestrol 1 mg 12 hourly for 48 hours prior to test.

Procedure

- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour before cannulating the patient.
- Using a glucose meter, determine the patient's fasting blood glucose concentration with a capillary blood sample obtained by finger prick.
- Take samples (time 0).
- If the fasting glucose meter result is <3.0 mmol/L – no glucagon is given, the patient is given oral glucose as described below and blood samples are collected according to the table up to 60 minutes
- If the fasting glucose meter result is ≥3.0 mmol/L - administer glucagon IM at time 0
  Dose: 15 µg/kg body weight to a maximum of 1 mg
- Continue the sampling using the table below.
  Ensure each sample and form is clearly marked with the actual collection time as well as 0, 30, 60 minutes etc.

	-30	0	30	60	90	120	150	180
Glucose (meter)	+	+	+	+	+	+	+	+
Glucose (lab)		+	+	+	+	+	+	+
GH		+	+	+	+	+	+	+
Cortisol		+	+	+	+	+	+	+
Grey top tubes		1	1	1	1	1	1	1
Gold top tubes		2	2	2	2	2	2	2

- The patient must be supervised throughout the procedure.
- **if at any time the glucose meter result is <3.0 mmol/L or if the child shows clinical signs of hypoglycaemia** (ie sweatiness, drowsiness) give 30 mL of oral glucose drink eg Hycal. This can be followed by breakfast.
  If the glucose meter result has not increased within 10-15 minutes give a further 30 mL oral Hycal.
  Continue to collect blood samples even though glucose has been given but ensure that the administration of glucose is clearly recorded in the notes and on the request form
- **If the child does not tolerate oral glucose or remains persistently hypoglycaemic** give IV glucose 200 mg/kg (ie 2 mL/kg 10% dextrose) over 3 minutes. This IV site cannot then be used for taking samples for glucose measurements. Commence IV glucose infusion at 2.4 – 4.8 mL/kg/h 10% dextrose (ie. 4 – 8 mg/kg/min glucose)
  Check the glucose meter result at 4 - 5 minutes and adjust glucose infusion (up to 6 mL/kg/h ie. 10 mg/kg/min glucose) to maintain blood glucose at 5 - 8 mmol/L and no higher.
- **if no response to IV glucose** give hydrocortisone 50 mg IV. Commence 10% dextrose infusion at 6 mL/kg/hr
- All symptoms must be documented in the patient notes.

- There is a risk of delayed hypoglycaemia: children should only be discharged once they have eaten and the glucose concentration is clearly normal.

Interpretation

An adequate cortisol response to exclude adrenal hypofunction is defined as a result greater than 480 nmol/L at any time point during the test.

An adequate GH response is a rise to a value greater than 6.7 µg/L at any time point during the test.

References

1. Barth et al. Biochemical Investigations in Laboratory Medicine http://www.pathology.leedsth.nhs.uk/dnn_bilm/Home.aspx (accessed 18-11-15)

13 LHRH test – protocol for paediatrics

Indications for Test

The LHRH test assesses pituitary and hypothalamic function. It is indicated in the assessment of precocious puberty, investigation of possible gonadotrophin deficiency or investigation of gonadal dysgenesis. This test can be combined with the glucagon test.

Patient Preparation

No patient preparation is required..

Side Effects

Warn patient of possible transient side effects – nausea, headache, abdominal pain.

Requirements

Serum (gold top) sample tube tubes for hormone measurements

LH releasing hormone (LHRH)

Procedure

- The child should be weighed on arrival and Emla cream applied to a suitable cannulation site, this should be allowed to stay in situ for at least 1 hour.
- Cannulate the patient and take baseline samples (-30 minutes).
- Take samples (time 0).
- Administer 2.5 µg/kg body weight LHRH IV (maximum 100 µg)
- Continue the sampling using the table below.

Analyte	**Time (minutes)**			
	-30	0	20	60
LH	+	+	+	+
FSH	+	+	+	+
Oestradiol	+			
Testosterone	+			
TSH	+			
FT4	+			
FT3	+			
Prolactin	+			
Gold top tubes	3	1	1	1

Interpretation

The results of pituitary function tests can be difficult to interpret. Interpretation must always be done in conjunction with clinical findings.

In prepubertal children LH concentration should peak at <5 IU/L with the FSH peak greater than LH.

In peripubertal and pubertal children there should be a greater response with the LH peak greater than FSH.

In children with suspected hypogonadotrophic hypogonadism a complete lack of response supports the diagnosis. However, measurable but low responses may be seen.

In primary gonadal failure both LH and FSH responses are exaggerated.

References

1. Barth et al. Biochemical Investigations in Laboratory Medicine http://www.pathology.leedsth.nhs.uk/dnn_bilm/Home.aspx (accessed 21-4-16)

14 Three day hCG stimulation test (basic) – protocol for paediatrics

Indications for Test

To determine whether testes are able to produce significant amounts of testosterone in response to stimulation by hCG (human chorionic gonadotrophin).

The test is usually performed in patients with NO palpable testes (in order to define whether testicular tissue is present or absent), or in patients who have undergone BILATERAL orchidopexy, in order to determine whether at least one of the previously undescended testes is able to produce testosterone in response to hCG.
N.B. This is the basic hCG test for indications as denoted above. For intersex disorders a more extensive baseline and post-hCG evaluation is required. There is considerable professional variation in accepted hCG test schedules.

Patient Preparation

None.

Procedure

- If LH, FSH and karyotype have been done previously they do not need repeating.
- Human chorionic gonadotrophin (hCG) is given im
- Dose of hCG is determined by age

<1 year	500 units/day
1-10 years	1000 units/day
>10 years	1500 units/day

- Serum testosterone is measured as shown in the table below.

	hCG Dose	Blood samples
Day 1	1st dose, after blood samples	Testosterone (LH, FSH, karyotyping)
Day 2	2nd dose	
Day 3	3rd dose	
Day 4		Testosterone

Sample Tubes

Testosterone	2 mL serum SST tube (gold top) e.g. x2 paediatric tubes
LH, FSH	gold top
Karyotyping	green top and Guys Genetics request form

Interpretation

There is a 2 to 9 fold increase in testosterone in normal prepubertal boys. Normal peak testosterone response is usually in the range of 2-8 nmol/L.

In the absence of testes there will be no change in testosterone concentration in response to hCG. Results are very variable and not readily predictive of long term testicular function.
If a reasonable response is observed, this can be reassuring until further assessment at an older age particularly around pubertal development (12-14 years old).

In some patients with very poor response to this short hCG rest, a prolonged hCG stimulation (e.g. 1000 IU x 2 / week for 6 weeks with serum testosterone measured 24 hours after last hCG dose can be useful to show evidence of possible gonadotrophin deficiency.

References

1. Great Ormond Street Hospital gonadal axis protocols.

15 3 week hCG stimulation test – protocol for paediatrics

Indications for test

This is generally used in patients with bilateral cryptorchidism in whom gonadotrophin deficiency (+ hyposmia/anosmia = Kallmann syndrome), anorchia ("vanishing testes") or a testosterone synthesis defect (5 alpha-reductase deficiency) are suspected. The test is usually combined with estimation of LH and FSH (with LHRH stimulation). The test has two purposes:

1. To stimulate the testes to produce testosterone over a prolonged period of time.
2. To facilitate testicular descent and to achieve an increase in the size of the phallus.

Patient Preparation

None.

Procedure

- Measure and record size of phallus and testes ± photo.
- Basal sample for measurement of serum testosterone and androgens. Check serum LH, FSH and karyotype if this has not been done.
- Human chorionic gonadotrophin (hCG) is given by IM injections twice weekly (Mon/Thurs or Tues/Fri) by Day Ward or GP for 3 weeks. For dosage of hCG see below:

< 1 year	500 units twice weekly
> 1-10 years	1000 units twice weekly
> 10 years	1500 units twice weekly

  The test can follow on from a 3 day hCG stimulation test: hCG is given after the blood samples on day 4 are taken, followed by a further 2 weeks of twice weekly injections.
- The patient should return to Day Ward for the post-hCG serum testosterone and androgen concentrations as shown in the table below:

Time	Testosterone	Androstenedione, DHEAS, dihydrotestosterone These tests are only required in children with hypospadias or ambiguous genitalia
Day 0, before first injection	+	+
Day 19, 24 hour after last injection	+	+
Gold top tubes	2	2

- Measure and record size of phallus and testes ± photo.

Interpretation

Interpretation of results should be undertaken by a consultant paediatrician and/or consultant endocrinologist.

References

1. Great Ormond Street Hospital gonadal axis protocols.

16 IGF-1 generation test

Indications for test

An IGF-1 generation test may be used for the diagnosis of growth hormone (GH) resistance syndromes (eg Laron-type dwarfism, LTD), idiopathic short stature with high GH and low IGF-1 suggesting partial GH insensitivity, the 'bioinactive' GH syndrome or neurosecretory dysfunction of GH secretion.

The patient should have had a GH provocation test (eg glucagon test) that showed a high peak GH concentration but with low IGF-1 and/or IGFBP3 concentrations for age and pubertal status.

Contraindications

None

Patient preparation

On day 1 and 5 the child should be fasted from midnight with only water to drink.

Procedure

- Fasting blood samples are collected in the morning on days 1 and 5.
- IGF-1 samples: 2mL serum (red top)
- Human growth hormone (hGH) is given SC in the evening on days 1, 2, 3 and 4.
  Dose: 0.03 mg/kg (ie 0.1 unit/kg)

	Time	hGH dose	Blood samples
Day 1	08:00 – 10:00	-	IGF-1
Day 1	16:00 – 19:00	1st dose	-
Day 2	16:00 – 19:00	2nd dose	-
Day 3	16:00 – 19:00	3rd dose	-
Day 4	16:00 – 19:00	4th dose	-
Day 5	08:00 – 10:00	-	IGF-1

Interpretation

In normal individuals IGF-1 concentrations increase by >20%.

In Laron-type dwarfism and partial GH insensitivity IGF-1 concentrations remain low for age.

In bioinactive GH and neurosecretory dysfunction there is a normal IGF-1 response to exogenous GH.

References

1. Great Ormond Street Hospital protocols.

17 Key Stakeholders, Consultation, Approval and Ratification Process

East Kent Hospitals University NHS Foundation Trust is the key stakeholder for this policy.

Consultation has been through e-mail and face-to-face communication between clinical biochemistry staff and Trust and visiting consultant paediatricians. Email correspondence is stored at S:\Path\SnrStaff\Comms with users\Clinical guidelines\Paediatric specialist investigations

18 Review and Revision Arrangements

Three years from implementation date, by author.

19 Dissemination and Implementation

SharePoint, by proactive implementation through the Divisions by appropriate clinical leads

20 Document Control including Archiving Arrangements

Archive of this document will be through QPulse.

21 Monitoring Compliance

Within the Trust, compliance with this policy must rest with the requesting Divisions with vetting of requests in Clinical Biochemistry. Compliance will also be subject to audit within Clinical Biochemistry.

22 References

See each protocol.

23 Associated Documentation

Not applicable

Appendix A - Equality Impact Assessment

Equality and Human Rights Impact Analysis (EHRIA)

Part One – Screening Tool

Name of the policy, strategy, function or methodology:	Paediatric Portfolio: Guidelines for specialist investigation

Details of person completing the EHRIA	
Name	Miss Elizabeth Hall
Job Title	Principal Clinical Scientist
Department/Specialty	Pathology/Clinical Biochemistry
Telephone Number	ext 722-2868

1. Identify the policy, strategy, function or methodology aims

What are the main aims, purpose and outcomes of the policy, strategy, function or methodology?
To ensure appropriate and consistent use of dynamic function test protocols in Paediatrics across the health service in East Kent.
Does it relate to our role as a service provider and/or an employer?
Service provider.

2. Assess the likely impact on human rights and equality

Use this table to check if the policy, strategy, function or methodology:

- could have a negative impact on human rights or on any of the equality groups, or
- could have a positive impact on human rights, contribute to promoting equality, equal opportunities or improve relations.

It is not necessary to complete each box, nor to mark whether it is positive or negative, although you can do this if you find it helpful.

	Protected Characteristic								
	Race	**Sex**	**Disability**	**Sexual Orientation**	**Religion or belief**	**Age**	**Gender reassignment**	**Marriage & Civil Partnership**	**Pregnancy & Maternity**
Could this policy, procedure, project or service affect this group differently from others? **YES/NO**									
Could this policy, procedure, project or service promote equal opportunities for this group? **YES/NO**									
Right to life *e.g. decisions about life-saving treatment, deaths through negligence in hospital*									
Right not to be tortured or treated in an inhuman or degrading way *e.g. dignity in care, abuse or neglect of older people or people with learning disabilities.*									
Right to respect for private and family life e.g. *respecting lgb relationships, confidentiality*									
Right to freedom of thought, conscience and religion *e.g. respect for cultural and religious requirements*									
Right to freedom of expression *e.g. access to appropriate communication aids*									
Right to freedom of assembly and association *e.g., right to representation, to socialise in care settings*									
Right to education *e.g. access to basic knowledge of hygiene and sanitation*									
Right to liberty e.g. informal detention of patients who do not have capacity									

3. How does it impact on people's human rights and equality?

Using the table above, explain anticipated impacts. If a full EHRIA is recommended, you can summarise the impacts - it is not necessary to set these out in detail,

Could people's human rights be impacted negatively? Could the policy, strategy, function or methodology result in inequality or discrimination?
No
Could this policy, strategy, function or methodology result in positive impacts on people's human rights or equality? Could it present opportunities to promote equality?
No

4. Recommendations

Is a full EHRIA recommended? If not, give reasons
No. The policy has equal impact.

5. Publication of EHRIA

Give details of where Screening Tool or the full EHRIA will be published and when this will take place
With document.

Details of person completing the EHRIA	
Name	Miss Elizabeth Hall, Principal Clinical Scientist

Signed …… Date: ……

Approval and sign-off	Name
Head of Department/Director	Dr Sally Stock, Head of Service Clinical Biochemistry

Signed …… Date: ……

	Name
Trust Board approval and sign-off	not applicable

Signed …… Date: ……

Appendix B – Author's Checklist of compliance with the Policy for the Development and Management of Organisation Wide Policies and Other Procedural Documents

POLICY:

To be completed and attached to any policy when submitted to the appropriate committee for consideration and approval.

	Requirement:	Compliant Yes/No/ Unsure	Comments
1.	**Style and format**	Yes	
2.	**An explanation of any terms used in documents developed**	Yes	
3.	**Consultation process**	Yes	
4.	**Ratification process**	Yes	
5.	**Review arrangements**	Yes	
6.	**Control of documents, including archiving arrangements**	Yes	
7.	**Associated documents**	n/a	
8.	**Supporting references**	Yes	
9.	**Relevant NHSLA criterion specific requirements**	n/a	
10.	**Any other requirements of external bodies**	n/a	
11.	**The process for monitoring compliance with NHSLA and any other external and/or internal requirements**	n/a	

Appendix C – Plan for Dissemination of Policies

To be completed and attached to any policy when submitted to the appropriate committee for consideration and approval.

Acknowledgement: University Hospitals of Leicester NHS Trust (Amended)

Title of document:	Paediatric investigations: guidelines for specialist biochemical tests		
Version Number:	2.0		
Approval Date:		**Dissemination lead:**	Miss Elizabeth Hall
Previous document already being used?	No		
If yes, in what format (paper / electronic) and where (e.g. Directorate / Trust wide)?			
Proposed instructions regarding previous document:	This guideline will replace all other documentation on how to perform dynamic function tests in Paediatrics		

To be disseminated to:	**How will it be disseminated, who will do it and when?**	**Format (i.e. paper or electronic)**	**Comments:**
Trust clinical staff	TrustNet	electronic	
Clinical Biochemistry staff	TrustNet, QPulse	electronic	Document to be circulated to staff on QPulse

Author's Dissemination Record - to be used once document is approved – to be kept with the master document

Date document forwarded to be put on the Trust's central register / in SharePoint:		**Date document put on Directorate register (if appropriate) / on Directorate webpage (if applicable)**	

Disseminated to: (either directly or via meetings, etc.)	**By Whom?**	**Format (i.e. paper or electronic)**	**Date Disseminated:**