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AUSTRALIA AND NEW ZEALAND HARMONISED PAEDIATRIC ENDOCRINE DYNAMIC TESTING MANUAL

Harmonisation of Endocrine Dynamic Testing in Paediatrics (HEDT-Paeds)

First Edition
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Table of contents

Introduction

Dynamic endocrine testing plays an integral role in the investigation of possible underlying endocrinopathies in paediatrics, but it is resource- and labour-intensive.

One of the greatest challenges to the effective utilisation of endocrine dynamic function tests is the significant variation in protocols adopted across various centres. This is no less apparent in Australia and New Zealand. Variations in the protocol for a particular endocrine dynamic test can range from differences in the dose or route of administration of the stimulating/inhibiting agent to differences in the timepoints for sampling to differences in the interpretation of results, with different cut-off thresholds being used to differentiate between what is deemed an appropriate 'normal' response and what is deemed a pathological 'abnormal' response. Patient and clinician factors, local department and healthcare system factors, assay factors, as well as time and access to the appropriate staffing, pharmacological agents, laboratory, and financial resources required to perform the endocrine dynamic tests can all be contributing factors underpinning the variation in protocols. A specific version of a protocol may have been created and written based on influences from the literature (evidence-based medicine), expert opinion, local resources and preferences, and/or challenges to implementation.

There are many advantages of harmonised protocols, including the accumulation of more data for prospective improvements in future protocols, the facilitation of research across centres, and the potential for the correlation of various cut-offs between assays. A recent collaborative initiative between the Endocrine Society of Australia (ESA), the Australasian Association for Clinical Biochemistry and Laboratory Medicine (AACB), and the Royal College of Pathologists Australasia (RCPA) has taken a positive step towards harmonising practice within Australasia with the publication of the *Harmonisation of Endocrine Dynamic Testing – Adult (HEDTA)* manual. This is a free, easily accessible, online resource which contains a comprehensive collection of endocrine dynamic function test protocols written for use by all centres across Australia and New Zealand that perform endocrine dynamic function testing in adults.

The Harmonisation of Endocrine Dynamic Testing in Paediatrics (HEDT-Paeds) project, a collaboration between the Australia and New Zealand Society for Paediatric Endocrinology and Diabetes (ANZSPED; previously known as the Australasia Paediatric Endocrine Group [APEG]), the AACB, and the RCPA, focuses on protocols commonly utilised in paediatrics.

The information provided in this *Australia and New Zealand Harmonised Paediatric Endocrine Dynamic Testing Manual* is intended as a guide only and needs to be verified by individual clinicians and laboratories prior to use. Modifications might be required according to local procedures (for example: patient consent, sample type, name of test set, collection, interpretation, and reporting procedures).

Methodology

As part of this HEDT-Paeds project, a survey of current paediatric endocrine dynamic testing practices was conducted to provide the authors of the HEDT-Paeds protocols with a better understanding of the current situation in different paediatric/paediatric endocrinology centres around Australia and New Zealand. This information was taken into consideration during the creation of the *Australia and New Zealand Harmonised Paediatric Endocrine Dynamic Testing Manual*. Where available, the current protocols used by major paediatric centres were reviewed.

The main tool used during the literature review was the University of Queensland Library's search engine. Sources were accessed through this search engine from databases including Web of Science, Scopus, PubMed Central (PMC), Directory of Open Access Journals, Ingenta Connect, Ovid, MEDLINE, ScienceDirect, Oxford Academic Journals, and Wiley Online Library. Medical journals available in the UQ Library and via online open access were also included in the search. Additional searches were performed through Google Scholar, the National Centre for Biotechnology Information (NCBI), PMC, and the Cochrane Library. Literature included for review was published between 2000 and 2022 and was written in English. Searches were kept strategically broad with the focus on the manual review of papers for relevance. Searches were limited to articles from the year 2000 onwards to incorporate protocol changes driven by developments in liquid chromatography-tandem mass spectrometry (LC-MS/MS) and next-generation sequencing (NGS) technology.

The literature was reviewed again when feedback from the draft version of the literature review was received following the consultation period, to determine whether there had been publication of any more relevant literature since the time of the review.

Based on the National Health and Medical Research Council (NHMRC) Levels of Evidence (see below), by the very nature of diagnostic testing, level I and level II categories of evidence were rare. Level III studies were often complicated by heterogeneity or differences in the definitions of the study population, a lack of a genetic confirmation of a condition, and limitations in the sensitivity and specificity of the assays used.

Table 1 National Health and Medical Research Council levels of evidence

Level	Intervention	Diagnosis	Prognosis	Aetiology	Screening
I	A systematic review of Level II studies	A systematic review of Level II studies	A systematic review of Level II studies	A systematic review of Level II studies	A systematic review of Level II studies
II	A randomised controlled trial	A study of test accuracy with an independent, blinded comparison with a valid reference standard, among consecutive patients with a defined clinical presentation	A prospective cohort study	A prospective cohort study	A randomised controlled trial
III-1	A pseudorandomised controlled trial (i.e. alternate allocation of some other method)	A study of test accuracy with an independent, blinded comparison with a valid reference standard, among consecutive patients with a defined clinical presentation	All or none	All or none	A pseudorandomised controlled trial (i.e. alternate allocation of some other method)
III-2	A comparative study with concurrent controls: • Non-randomised, experimental trial • Cohort study • Case-control study • Interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1	Analysis of prognostic factors amongst untreated control patients in a randomised controlled trial	A retrospective cohort study	A comparative study with concurrent controls: • Non-randomised, experimental trial • Cohort study • Case-control study
III-3	A comparative study without concurrent controls: • Historical control study • Two or more single arm study • Interrupted time series without a parallel control group	Diagnostic case-control study	A retrospective cohort study	A case-control study	A comparative study without concurrent controls: • Historical control study • Two or more single arm study
IV	Case studies with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard)	Case series, or cohort study of patients at different stages of disease	A cross-sectional study	Case studies

In the absence of definitive evidence for the adoption of changes to specific protocols, a comparison was made between currently used protocols and a pragmatic decision was made to incorporate similarities in the recommended protocols for adoption in the *Australia and New Zealand Harmonised Paediatric Endocrine Dynamic Testing Manual*.

Section I

Assessment of disorders of growth

GROWTH HORMONE STIMULATION TEST (GHST)
Stimulant: Arginine

Indication:

To test growth hormone (GH) release from the anterior pituitary gland in individuals being assessed for growth hormone deficiency (GHD).

Rationale:

The hypothalamus stimulates the release of GH from somatotropes in the anterior pituitary gland via GH-releasing hormone (GHRH). Secretion of GH subsequently stimulates insulin-like growth factor 1 (IGF-1) production in the liver. Both GH and IGF-1 play important roles in promoting linear growth. Evaluation of this response is important in the evaluation of disorders of growth.

Arginine stimulates GH secretion by (1) stimulation of α-adrenergic receptors with subsequent GHRH release and (2) suppression of somatostatin.

Contraindications:

Severe renal, cardiac, or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Current acute illness.

Untreated hypothyroidism (thyroxine deficiency may reduce GH response).

Certain drugs, for example, cyproheptadine (Periactin), which interfere with arginine stimulation (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Known allergic tendencies.

Precautions:

Ensure the patient has robust intravenous access for arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulus to the pituitary gland and nullification of the GH stimulation test.

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation and dose:

Table 2 Arginine hydrochloride dose and route

Formulation	Dose	Route
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Adverse reactions:

Rapid intravenous infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis are extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after administration of arginine infusion.

Elevated potassium levels have been observed in uraemic patients.

There have been case reports of transient haematuria following arginine stimulation tests.

Children may experience hypoglycaemia. This can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine as deaths have been reported with dilution errors (there have been cases where patients have received 10 times the intended dose).

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the GHST.

Ensure the patient has normal electrolyte results prior to commencing the GHST.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If the patient is already on growth hormone, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming

in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

Table 3 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronised estradiol valerate	Weight ≤20kg: 1 mg once daily orally Weight >20kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – arginine

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps outlined in the Preparation section have been undertaken prior to proceeding with the test.
2. Weigh the patient, calculate the arginine dose, and take the baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer the arginine via intravenous infusion over 30 minutes. The time that the infusion commences (not finishes) is Time 0. Allow time to give a 10–15-mL flush with sodium chloride 0.9% prior to taking the 30-minute blood sample.
5. Conduct the blood sampling, as below. If performed as part of a combined pituitary test, see combined protocol.

6. Check the blood glucose level using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After correction of hypoglycaemia, continue with collecting the remaining samples for the stimulation test.
7. No food (other than for treatment of hypoglycaemia) is to be ingested until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level to be discharged. If abnormal, repeat as required. Review by medical or nursing personnel (as per local practice) prior to discharge.

Sample collection:

Table 4 Arginine administration and sample collection summary

Drug Administered:			**Dose Administered:**	**Time Administered:**			
Actual time bloods taken:	Baseline	Administer arginine	Minutes post START of arginine infusion				
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min
GH	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓
Other tests, e.g., IGF-1, IGFBP-3, ACTH cortisol, as per requesting clinician	+/-						
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube

Interpretation:

The GH level used as the cut-off threshold for diagnosing and treating GHD varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, different criteria must be met, and these are determined by the Pharmaceuticals Benefits Scheme (PBS) in Australia or by Pharmac in New Zealand. Please

check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 5 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGFPB-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test

Table 6 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GH-deficient sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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GROWTH HORMONE STIMULATION TEST (GHST)
Stimulant: Glucagon

Indication:

To test growth hormone (GH) release from the anterior pituitary gland in individuals being assessed for growth hormone deficiency (GHD).

Please note, glucagon stimulation of the hypothalamic-pituitary-adrenal axis is not robust.

Rationale:

The hypothalamus stimulates the release of GH from somatotropes in the anterior pituitary gland via GH-releasing hormone (GHRH). Secretion of GH subsequently stimulates insulin-like growth factor 1 (IGF-1) production in the liver. Both GH and IGF-1 play important roles in promoting linear growth. Evaluation of this response is important in the evaluation of disorders of growth.

Glucagon stimulates GH secretion by stimulation of the α-adrenergic receptors with subsequent GHRH release. Glucagon also causes a rise in blood glucose levels and subsequent insulin secretion which indirectly stimulates GH and adrenocorticotropic hormone (ACTH) secretion.

Contraindications:

Recent or intercurrent illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce GH and cortisol response)

Diabetes (glucagon stimulation test is unreliable in individuals with diabetes as this GH ‘stimulus’ requires endogenous insulin)

Patients who have not eaten for 48 hours, who have a glycogen storage disorder (GSD), or who have severe cortisol deficiency (in these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur).

Certain drugs which interfere with glucagon stimulation (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Precautions:

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation and dose:

Table 1 Glucagon formulation and dose

Formulation	Dose
Glucagon hydrochloride (1 mg; powder + diluent)	30 mcg/kg subcutaneously (max 1 mg)

Adverse reactions:

Transient nausea, flushing, vomiting for 1–2 minutes, abdominal pain/cramps, and/or a feeling of apprehension may occur.

Glucagon stimulates a 2–3-fold rise in blood glucose level following administration. This is maximal within the first hour. Following this rise in blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Anaphylaxis is a very rare, but potential, complication.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If the patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

Table 2 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20 kg: 1 mg once daily orally Weight >20 kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – glucagon

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps outlined in the Preparation section have been undertaken prior to proceeding with the test.
2. Weigh the patient, calculate the glucagon dose, and take the baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer the glucagon subcutaneously or intramuscularly, as per the dosing table above.
5. Conduct the blood sampling, as below. If performed as part of a combined pituitary test, see the combined protocol.
6. Check the blood glucose level using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. Consider giving an oral glucose drink if there has been a downward trend and the blood glucose level is <3.2 mmol/L, to help maintain adequate glucose levels. Hypoglycaemia corrected with an oral glucose drink will not compromise the interpretation of the test results. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.

7. No food (other than for treatment of hypoglycaemia) is to be ingested until the test is completed. Water is permitted.

Discharge:

For discharge, the child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 3 Glucagon administration and sample collection summary

Drug Administered:		**Dose Administered:**			**Time Administered:**		
	Minutes pre-glucagon	**Administer glucagon**	**Minutes post-glucagon injection**				
Actual time bloods taken:							
Test	-1 Min		60 Min	90 Min	120 Min	150 Min	180 Min
GH	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓
Other tests, e.g., IGF-1, IGFBP-3, ACTH, cortisol at baseline and various timepoints, as per requesting clinician	+/-						
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 2 mL	SST 2 mL	SST 2 mL	SST 2 mL	SST 2 mL

Note. ACTH, adrenocorticotropic hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube

Interpretation:

The GH level used as the cut-off threshold for diagnosing and treating GHD varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the Pharmaceuticals Benefits Scheme (PBS) in Australia or by Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 4 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGFPB-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test

Table 5 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GH stimulation tests. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

See the Short Synacthen Test protocol for interpretation of cortisol levels.

Please note that the specificity of the GST for diagnosing cortisol deficiency is low, that is, a suboptimal cortisol response does not confirm deficiency.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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GROWTH HORMONE STIMULATION TEST
Stimulant: Clonidine

Indication:

To test growth hormone (GH) release from the anterior pituitary gland in individuals being assessed for growth hormone deficiency (GHD).

Rationale:

The hypothalamus stimulates the release of GH from somatotropes in the anterior pituitary gland via GH-releasing hormone (GHRH). Secretion of GH subsequently stimulates insulin-like growth factor 1 (IGF-1) production in the liver. Both GH and IGF-1 play important roles in promoting linear growth. Evaluation of this response is important in the evaluation of disorders of growth.

Clonidine stimulates GH secretion by (1) stimulation of the α-adrenergic receptors with subsequent GHRH release, and (2) suppression of somatostatin.

Contraindications:

Sick sinus syndrome, compromised intravascular volume, hypotension, syncope, autonomic dysfunction, or recent or intercurrent illness

Untreated adrenal insufficiency, hypothyroidism, or panhypopituitarism.

Children with known congenital/acquired heart disease (exercise caution)

Certain drugs which interfere with clonidine stimulation (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Expertise level:

The minimum requirement is for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation and dose:

Table 1 Clonidine formulation and dose

Formulation	Dose	Notes
Clonidine tablet	100 micrograms/m2 orally (maximum 250 micrograms)	Calculate dose to nearest half tablet

Note:

Clonidine 100-microgram and 150-microgram tablets available on the Pharmaceutical Benefits Scheme (PBS) in Australia.

Clonidine 25-microgram and 150-microgram tablets are available in New Zealand.

Adverse reactions:

Drowsiness 1–3 hours post ingestion, nausea, and/or vomiting.

Hypotension and/or postural hypotension marked by a fall in blood pressure of approximately 10 mm Hg about 1 hour after ingestion which usually resolves by the end of the test but may last several hours. The effect may be prolonged in renal failure. 10 mL/kg 0.9% sodium chloride bolus given over 30 minutes following clonidine administration can minimise the fall in blood pressure.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the clonidine stimulation test.

If the patient is on regular antihypertensive medication, please check with the consultant responsible for the patient about withholding this medication prior to the test.

If the patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the growth hormone stimulation test (GHST).

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on request form.

Note: children with ADHD may be on clonidine treatment doses of up to 400 mcg daily. The clonidine should be withheld the day before and the day of the test. Consideration should be given to using an alternative protocol, such as arginine or glucagon.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

Table 2 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20 kg: 1 mg once daily orally Weight >20kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for intravenous (IV cannulation) and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – clonidine

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps outlined in the Preparation section have been undertaken prior to proceeding with the test. Ideally, perform the test first thing in the morning following an overnight fast (see fasting protocol for age-based maximum fasting durations). However, a minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh the patient, calculate the clonidine dose, and take the baseline observations.
3. Ensure the child is recumbent and resting during the test. The child can drink water during the test. No food is to be ingested until the test is completed.
4. Insert the IV cannula and take baseline (pre-stimulation) blood samples. Flush the IV cannula with 0.9% sodium chloride.
5. Administer the clonidine orally (with water), as per the dosing table above.
6. Give an IV bolus of 10 mL/kg of 0.9% sodium chloride over 30 minutes following clonidine administration to minimise the fall in blood pressure. **Note, the clinician may choose to give a volume less than 10 mL/kg, depending on the size/age of the child.
7. The timing of further blood sampling is provided in the table below. If performed as part of a combined pituitary test, see the combined protocol.
8. Check the blood glucose level using a bedside glucometer/point-of-care machine at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
9. For symptomatic hypotension during the test (>30% fall in systolic BP from the pre-test systolic BP or a systolic BP <80 mmHg) consider a further bolus of 10 mL/kg 0.9% sodium chloride. If unsure or no response, call the medical team for advice.
10. Take care ambulating the child following completion of the test as postural hypotension may occur.
11. No food (other than for the treatment of hypoglycaemia) is to be ingested until the test is completed. Water is permitted.

Discharge:

For discharge, the child must have been fed, have normal observations and blood glucose level, and have been observed for a minimum of 30 minutes following completion of the test. If observations are abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 3 Clonidine administration and sample collection summary

Drug Administered:		**Dose Administered:**			**Time Administered:**		
	Baseline	**Administer Clonidine**	**Minutes post-clonidine**				
Actual time bloods taken:							
Test	-1 Min		30 Min	60 Min	90 Min	120 Min	150 Min*
GH	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓
Other tests, e.g., IGF1, IGFBP3, as per requesting clinician							
Sample Tubes / Minimum Blood Volume							
*Optional collection timepoint							

Note. ACTH, adrenocorticotropic hormone; GH, growth hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating GHD varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the PBS in Australia) or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 4 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGFPB-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test

Table 5 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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GROWTH HORMONE STIMULATION TEST
Combined Protocol
Stimulants: Glucagon and Arginine

Indication:

To test growth hormone (GH) release from the anterior pituitary gland in individuals being assessed for growth hormone deficiency (GHD).

Please note, glucagon stimulation of the hypothalamic-pituitary-adrenal axis is not robust.

Rationale:

The hypothalamus stimulates the release of GH from somatotropes in the anterior pituitary gland via growth hormone releasing hormone (GHRH). Secretion of GH subsequently stimulates insulin-like growth factor 1 (IGF-1) production in the liver. Both GH and IGF-1 play important roles in promoting linear growth. Evaluation of this response is important in the evaluation of disorders of growth.

Arginine stimulates GH secretion by (1) stimulation of the α-adrenergic receptors with subsequent GHRH release and (2) suppression of somatostatin. It also causes a rise in blood glucose levels with subsequent insulin secretion which indirectly stimulates GH and ACTH secretion.

Contraindications:

Severe renal, cardiac, or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce GH and cortisol response)

Patients who have not eaten for 48 hours, who have a glycogen storage disorder (GSD), or who have severe cortisol deficiency (in these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur).

Diabetes (the glucagon stimulation test is unreliable in individuals with diabetes as this GH 'stimulus' requires endogenous insulin)

Certain drugs, for example, cyproheptadine (Periactin), which interfere with arginine stimulation.

Certain drugs which interfere with glucagon and/or arginine (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Known allergic tendencies.

Precautions:

Ensure the patient has robust (IV) access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation of the pituitary gland and nullification of the GH stimulation test.

Any urine testing for amino acids <24 hours after the arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation and dose:

Table 1 Arginine formulation and dose

Formulation	**Dose**	**Route**
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	IV infusion over 30 minutes

Table 2 Glucagon formulation and dose

Formulation	**Dose**
Glucagon hydrochloride (1 mg; powder + diluent)	30 mcg/kg subcutaneously (maximum 1 mg)

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and anaphylaxis are extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after an arginine infusion has been administered.

Elevated potassium levels in uraemic patients.

There have been case reports of transient haematuria following arginine stimulation tests.

Children may experience hypoglycaemia. This can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine is administered, as deaths have been reported with dilution errors, with some patients having received 10 times the intended dose.

Glucagon

Transient nausea, flushing, vomiting for 1–2 minutes, abdominal pain/cramps, and feelings of apprehension may occur.

Glucagon stimulates a 2–3-fold rise in blood glucose level following its administration. This increase is maximal within the first hour. Following this rise in blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Anaphylaxis is a very rare, but potential, complication.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the growth hormone stimulation test (GHST).

Ensure patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If the patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires priming of all children at the age of 5–6 years for GH stimulation testing.

Table 3 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20kg: 1 mg once daily orally Weight >20kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2 mL and 5 mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – glucagon, arginine

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps outlined in the Preparation section have been undertaken prior to proceeding with the test.
2. Weigh the patient, calculate the arginine and glucagon doses, and take the baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer the glucagon subcutaneously (dose as per dosing table above).
5. Administer the arginine via IV infusion over 30 minutes. Time at 180 minutes is measured as the time from when the infusion **starts** (not finishes). Ensure to flush with 10–15 mL of sodium chloride 0.9% prior to taking the 210-minute blood sample.
6. Blood sampling as per the table below.
7. Check the blood glucose level using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
8. No food (other than for treatment of hypoglycaemia) until the test is completed. Water is permitted.

Discharge:

For discharge, the child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 4 Glucagon and arginine administration, dose, and sampling summary

	Drug Administered			**Dose Administered**			**Time Administered**				
	Baseline		Minutes post START of glucagon injection								
Actual time bloods taken		Administer glucagon					Administer arginine				
Test	-1 Min		60 Min	90 Min	120 Min	150 Min	180 Min		210 Min	225 Min	240 Min	255 Min	270 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Other tests, e.g., IGF-1, IGFBP-3, ACTH, cortisol at baseline and various timepoints, as per requesting clinician	+/-												
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube. **Optional samples for the study investigating the diagnostic utility of glucagon-stimulated copeptin in polyuria-polydipsia syndrome.

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating GHD varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by the PBS in Australia or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 5 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGFPB-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test

Table 6 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (e.g., cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

See the Short Synacthen Test protocol for interpretation of cortisol levels.

Please note that the specificity of the GST for diagnosing cortisol deficiency is low, that is, a suboptimal cortisol response does not confirm deficiency.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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GROWTH HORMONE STIMULATION TEST
Combined Protocol
Stimulants: Arginine and Clonidine

Indication:

To test growth hormone (GH) release from the anterior pituitary gland in individuals being assessed for growth hormone deficiency (GHD).

Rationale:

The hypothalamus stimulates release of GH from somatotropes in the anterior pituitary gland via GH-releasing hormone (GHRH). Secretion of GH subsequently stimulates insulin-like growth factor 1 (IGF-1) production in the liver. Both GH and IGF-1 play important roles in promoting linear growth. Evaluation of this response is important in the evaluation of disorders of growth.

Arginine and clonidine stimulate GH secretion by (1) stimulation of the α-adrenergic receptors with subsequent GHRH release, and (2) suppression of somatostatin.

Contraindications:

Severe renal, cardiac, or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated hypothyroidism, adrenal insufficiency, or panhypopituitarism.

Certain drugs, for example, cyproheptadine (Periactin), which interfere with arginine stimulation

Certain drugs which interfere with arginine and/or clonidine (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Known allergic tendencies.

Sick sinus syndrome, compromised intravascular volume, hypotension, syncope, autonomic dysfunction, or recent or intercurrent illness.

Children with known congenital/acquired heart disease (exercise caution).

Precautions:

Ensure the patient has robust intravenous (IV) access for arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation and dose:

Table 1 Arginine formulation and dose

Formulation	Dose	Route
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	IV infusion over 30 minutes

Table 2 Clonidine formulation and dose

Formulation	Dose	Route	Notes
Clonidine	100 micrograms/m2 (maximum 250 micrograms)	Oral	Calculate dose to nearest half tablet

Note

Clonidine 100-microgram and 150-microgram tablets are available on the PBS in Australia.

Clonidine 25-microgram and 150-microgram tablets are available in New Zealand.

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and anaphylaxis are extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after an arginine infusion has been administered.

Elevated potassium levels in uraemic patients.

There have been case reports of transient haematuria following arginine stimulation tests.

Children may experience hypoglycaemia. This can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine is administered, as deaths have been reported with dilution errors, with some patients having received 10 times the intended dose.

Clonidine

Drowsiness 1–3 hours post ingestion, nausea, and vomiting.

Hypotension and/or postural hypotension marked by a fall in blood pressure of approximately 10 mm Hg about 1 hour after ingestion which usually resolves by the end of the test but may last several hours. The effect may be prolonged in renal failure. 10 mL/kg 0.9% sodium chloride bolus given over 30 minutes following clonidine administration can minimise the fall in blood pressure.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the GHST.

Ensure the patient has normal electrolytes prior to commencing the GHST.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If patient is already on growth hormone, this should ideally be ceased at least 96 hours (daily rhGH) or four weeks (weekly rhGH) prior to the GHST.

If the patient is on regular antihypertensive medication, please check with the consultant responsible for the patient about withholding this medication prior to the GHST.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Note: children with ADHD may be on clonidine treatment doses of up to 400 mcg daily. The clonidine should be withheld the day before and the day of the test. Consideration should be given to using an alternative protocol, such as arginine or glucagon.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of estrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for growth hormone stimulation testing.

Table 3 Sex steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20kg: 1 mg once daily orally Weight >20kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, clonidine

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test unless otherwise stipulated by the consultant or local policy.

Method:

1. Ensure the steps outlined in the Preparation section have been undertaken prior to proceeding with the test. Ideally, perform test first thing in the morning following an overnight fast (see fasting protocol for age-based maximum fasting durations). However, a minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh the patient, calculate the arginine and glucagon doses, and take the baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer the arginine via IV infusion over 30 minutes. The time that the infusion **starts** (not finishes) is regarded as Time 0. Allow time to give a 10–15 mL flush with sodium chloride 0.9% prior to taking the 30-minute blood sample.
5. Administer the clonidine orally (dose as per dosing table above) as soon as the +90-Min blood sample has been collected.
6. Consider giving an IV bolus of 10 mL/kg 0.9% sodium chloride over 30 minutes following the clonidine administration to minimise the fall in blood pressure. **Note: The clinician may choose to give a volume less than 10 mL/kg depending on the volume administered at the time of the arginine infusion and the size/age of the child.
7. Blood sampling as per the table below.
8. Check the blood glucose level using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
9. For symptomatic hypotension during the test (> 30% fall in systolic BP from the pre-test systolic BP or a systolic BP <80 mmHg), consider a further bolus of 10 mL/kg 0.9% sodium chloride. If unsure or no response, call the medical team for advice.
10. Take care ambulating the child following completion of the test as postural hypotension may occur.
11. No food (other than for the treatment of hypoglycaemia) is to be ingested until the test is completed. Water is permitted.

Discharge:

For discharge, the child must have been fed, have normal observations and blood glucose level, and have been observed for a minimum of 30 minutes following completion of the test. If observations are abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 4 Arginine and clonidine administration, dose and sampling summary

Drug Administered			**Dose Administered**						**Time Administered**				
	Baseline		Minutes post START of arginine infusion										
Actual time bloods taken		Administer arginine						Administer clonidine					
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	240 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Cortisol	✓		✓		✓								
ACTH	✓												
Other tests, for example, IGF-1 and IGFBP-3, as per requesting clinician	+/-												
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating GHD varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by the Pharmaceutical Benefits Scheme (PBS) in Australia) or Pharmac in New Zealand.

Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 5 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGFPB-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test

Table 6 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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ORAL GLUCOSE TOLERANCE TEST
for investigation of growth hormone excess

Indications:
To assess for growth hormone (GH) excess in individuals with suspected gigantism or acromegaly.

Rationale:

GH-releasing hormone (GHRH) from the hypothalamus stimulates the production of GH by somatotrophs in the anterior pituitary which subsequently stimulates the synthesis of insulin-like growth factor 1 (IGF-1) in the liver. Excess amounts of circulating GH and IGF1 give rise to gigantism (in individuals with open physes) or acromegaly (in individuals who have undergone physeal fusion). In normal physiological conditions, GH is suppressed by glucose.

GH secretion (amplitude and frequency) and suppressibility differ across pubertal stages, necessitating the use of Tanner-specific cut-offs for determining appropriate GH suppression following a glucose load.

There is evidence that GH suppression in pubertal tall children following a 75-g glucose load is less than that observed in adults. Adoption of a protocol based on a 100-g glucose load will ensure adequate and more consistent GH suppression, regardless of pubertal stage.

Contraindications:

Fasting hyperglycaemia >10 mmol/L on glucose meter (consider terminating the test).

Overt diabetes (symptomatic or random plasma glucose ≥11.1 mmol/L on two occasions).

Intercurrent illness, e.g., infection (the test is invalid in the presence of intercurrent illness which can impair glucose tolerance).

Recent surgery or trauma which may impair glucose tolerance.

Beta-blockers, corticosteroids, phenytoin, thiazides, or oestrogens, which can impair glucose tolerance (caution should be taken – it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Expertise level:

The minimum requirement for the Oral Glucose Tolerance Test (OGTT) is that it be performed in a centre with laboratory staff familiar with paediatric laboratory testing.

Formulation:

Oral glucose solution (centre-specific formulation).

Commercial glucose preparations (many containing partially hydrolysed starch) are often used in the OGTT. Potential differences between anhydrous and monohydrate forms of glucose in the OGTT have not been sufficiently elucidated.

Dose:

2.35 g/kg body weight of glucose dissolved in water, to a maximum of 100 g (body weight ≥43kg), consumed within 10 minutes.

Adverse reactions:

About 15% of patients are unable to tolerate glucose solutions, suffering from nausea and vomiting.

Occasionally, patients experience rebound hypoglycaemia towards the end of the test, with sweating and pallor.

Preparation:

Unrestricted diet with adequate carbohydrate intake for age (in adults: at least 150g carbohydrates per day) for at least three days before the test. This is because carbohydrate restriction can falsely elevate glucose levels in an OGTT.

Normal physical activity, no intercurrent illness.

The test should be performed in the morning after a 10–16-hour overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood sampling –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- access to hypoglycaemia treatment supplies (see Notes section below)

Observations:

On arrival, measure the patient's blood pressure (BP), pulse, weight, and height.

Observe the blood glucose level via glucometer on each blood sample.

Method:

1. Weigh the patient and take baseline observations.
2. Calculate and measure out the volume of glucose solution to be consumed (if not already pre-prepared).
3. Insert the IV cannula.
4. Collect baseline (pre-stimulation) bloods and measure the glucose level with a bedside/point-of-care glucometer.
5. The glucose drink is to be consumed over **no more** than 10 minutes.
6. Emphasise to the patient that he/she is to be resting during the test. Water is permitted.

7. Blood samples are to be collected at the timed intervals as per the table below and the glucose level is to be also measured using the bedside/point-of-care glucometer at each sampling time point. Blood samples are timed from the moment of the first swallow, which is defined as time 0 minutes.
8. The patient is to be fed before discharge. Remove the IV cannula when diet and fluids are tolerated.

Discharge:

For discharge, the child must have eaten and have a normal blood glucose level. All observations should be within normal limits; if abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 1 Glucose administration and sample collection summary

	Baseline	**Oral glucose load**	**Time post glucose load**					
Actual time bloods taken								
Test	**-1 Min**		**30 Min**	**60 Min**	**90 Min**	**120 Min**	**150 Min**	**180 Min**
Glucose	✓		✓	✓	✓	✓	✓	✓
GH	✓		✓	✓	✓	✓	✓	✓
IGF1	✓							
Other tests, for example, HbA1c, insulin, c-peptide, as per consultant responsible for patient	+/-		+/-	+/-	+/-	+/-	+/-	+/-
Sample tubes / Minimum blood volumes								

Note. GH, growth hormone; IGF-1, insulin-like growth factor 1; HbA1c, glycosylated haemoglobin; Minimal paediatric data is available for the use of the 150-min and 180-min blood samples; if time constraints or difficulties are experienced in obtaining the blood samples, the 150-min and 180-min post glucose load blood samples can be omitted.

Interpretation:

The original GH cut-off of <1.0 mcg/L was established using older immunoradiometric assays. Using more sensitive immunoassays, a GH cut-off of <0.3 mcg/L has been established in adults, but this is likely lead to some false positive results based on the limited paediatric data available.

Specific male and female GH cut-offs based on the Tanner stages have been proposed (refer to the table below).

Table 2 Male and female GH cut-offs based on the Tanner stages (Misra et al., 2007)

	Mean nadir GH (mcg/L)	Range (mean –2 SD to mean + 2 SD) nadir GH (mcg/L)	Minutes post glucose load to reach nadir
Female			
Tanner 1	0.09	0.03–0.23	60
Tanner 2–3	0.22	0.03– .57	60
Tanner 4–5	0.16	0.04–0.64	30
Male			
Tanner 1–2	0.10	0.03–0.39	90
Tanner 3–4	0.21	0.09–0.48	90
Tanner 5	0.10	0.02–0.50	90

Note:

Cut-offs were generated in children 9–17 years.

There are some individuals who do not exhibit pathological GH excess (gigantism, acromegaly) but may fail to suppress their GH levels during an OGTT. Situations where this may occur include adolescence, reactive hypoglycaemia, chronic renal failure, liver failure, active hepatitis, anorexia nervosa, malnutrition, hyperthyroidism, and diabetes.

Blood tubes/minimum collection volume

Please confirm with your local laboratory the blood tubes and minimum blood volumes that are required to run the tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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Section II

Assessment of disorders of puberty and gonadal function

GONADOTROPHIN RELEASING HORMONE (GnRH) STIMULATION TEST for the assessment of disorders of puberty

Indications:

Investigation of early activation of the hypothalamic-pituitary-gonadal (HPG) axis – precocious puberty.

Investigation of delayed activation of the HPG axis – constitutional delay vs hypogonadotropic hypogonadism (HH).

Rationale:

Gonadotrophin-releasing hormone (GnRH), secreted by the hypothalamus, stimulates the release of the gonadotropins – luteinising hormone (LH) and follicle-stimulating hormone (FSH) – from the anterior pituitary gland. The pattern of gonadotropin release following stimulation using a GnRH agonist is used to assess the activation and function of the HPG axis.

There is limited evidence of the efficacy of the GnRH stimulation test to distinguish delayed puberty from HH. The diagnostic utility of anti-Müllerian hormone (AMH) and inhibin B is becoming more recognised, although this has not been completely elucidated.

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay-specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs before relying on baseline LH levels for the assessment of precocious puberty.

Contraindications:

Pregnancy (relative contraindication).

Certain drugs which interfere with GnRH stimulation (it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and intravenous (IV) cannulation skills.

Formulation and dose:

Table 1 GnRH agonist formulations and doses

Formulation	Dose	Route
Australia		
Triptorelin acetate solution (Decapeptyl 100 micrograms/mL) Note: DO NOT USE Diphereline depot injection (long-acting triptorelin)	100 micrograms/m2 (max 100 micrograms)	Subcutaneous
New Zealand		
Gonadorelin (HRF, Ayerst, Factrel)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)

Note. These GnRH agonist formulations are the ones currently most easily accessible in each country.

Adverse reactions:

Significant adverse reactions have not been encountered. Occasionally, patients may experience nausea, headache, and abdominal pain.

Preparation:

The GnRH stimulation test can be used in combination with other stimulation tests as part of the assessment of pituitary function. When combined with a growth hormone stimulation test, sex-steroid priming is not necessary.

This test can be performed at any time of the day. The patient does not need to be fasting.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – triptorelin OR gonadorelin

Observations:

Observe the temperature, blood pressure (BP), and heart rate (HR) at baseline and then hourly throughout the test**,** unless stipulated otherwise by the consultant or local policy.

Method:

1. Weigh the patient and take baseline observations.
2. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
3. Administer the GnRH agonist (dose/route as per table above).
4. Blood sampling, as below. If performed as part of a combined pituitary test, see the combined protocol.
5. Remove the IV cannula once testing is complete.

Note:

If IV cannulation is not feasible and your unit has a subcutaneously administered GnRH agonist available to use as the stimulant, then bloods can be collected via venepuncture or finger prick/heel prick. At a minimum, bloods need to be collected at baseline and one timepoint following administration of the GnRH agonist. See 'Notes' section below for further details on the timing of post-GnRH agonist stimulation blood sampling.

Discharge:

Once the test is complete, ensure the patient meets the discharge criteria as per your local unit. If a 24-hour post-GnRH agonist blood test has been requested, ensure that arrangements have been made for this.

Sample collection:

Table 2 GnRH agonist administration and sample collection summary

Drug Administered:			**Dose Administered:**				**Time Administered:**		
Actual time bloods taken:		Baseline	Administer triptorelin OR gonadorelin	Minutes post triptorelin OR gonadorelin administration					
Test		-1 Min		30 Min	45 Min	60 Min	120 Min	180 Min	24** Hours
LH and FSH	triptorelin used	✓		✓	-	✓	✓	✓	-
	gonadorelin used	✓		✓	✓	✓	-	-	-
Testosterone (males) Estradiol (females)***		✓		-	-	-	-	-	✓
Blood Tubes / Minimum Blood Volume*		SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Notes. FSH, follicle-stimulating hormone; LH, luteinising hormone; SST, serum separator tube; *See Notes section below (regarding the timing of post-GnRH agonist stimulation blood samples); **For diagnostic workup of delayed puberty; ***It is preferable to use a liquid chromatography-tandem mass spectrometry method for the oestradiol measurement. This can be ordered for send-away testing if not available in the local laboratory.

Interpretation:

An LH peak post-GnRH agonist of ≥5.0 IU/L with an LH-dominant response suggests HPG axis activation. This LH cut-off is the most widely accepted in the literature but is dependent on the assay used.

An LH peak post-GnRH agonist of <5.0 IU/L with an FSH-dominant response is supportive of premature thelarche which may warrant continued monitoring of pubertal progression.

See 'Notes' section below regarding the use and interpretation of the GnRH stimulation test for the diagnosis of precocious puberty in children younger than 3 years old.

A complete lack of a gonadotropin response supports the diagnosis of HH, whereas a measurable but low response has limited predictive value (and may also occur in the constitutional delay of puberty).

Notes:

Blood tubes / minimum blood volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Effect of sex and/or Tanner stage on GnRH stimulation test results

Girls with signs of early puberty (Tanner stages 2–3) who undergo a GnRH stimulation test as part of the assessment for central precocious puberty (CPP) may reach a reasonably low-peak LH level during the GnRH stimulation test, while girls with CPP who have more advanced signs of puberty (Tanner stages >3) and boys with CPP tend to have a brisker LH response. In the girls with early puberty, additional measures from the GnRH stimulation test that may assist with differentiating between CPP and idiopathic premature thelarche (IPT) are a peak LH/peak FSH ratio above a certain threshold and/or a 24-hour post-GnRH stimulation oestradiol level in the pubertal range.

Timing of post-triptorelin/gonadorelin blood sampling note

Peak LH response has been reported to occur at various time points between 30 minutes to 180 minutes post-GnRH/GnRH agonist stimulation. This is dependent on the study design, the GnRH/GnRH agonist used, the sampling timepoints used, and the LH assay used.

If only taking blood samples at baseline and 1–2 timepoint post-GnRH/GnRH agonist stimulation due to time constraints or because of challenges with collecting multiple blood samples, then, according to the available literature, the best times to take the stimulated LH sample(s) (i.e. the timepoint(s) with the best diagnostic accuracy for central precocious puberty) are:

Triptorelin studies: LH sample taken at either 30 min, 60 min, or 180 min post-triptorelin

Gonadorelin studies: LH sample taken at either 30 min, 40 min, 45 min, or 60 min post-gonadorelin

Please confirm with the consultant responsible for the patient the timepoints they would like samples to be taken.

Some studies support the additional sampling timepoint of 24 hours post-GnRH/GnRH agonist stimulation for a testosterone/oestradiol level to improve the diagnostic accuracy of the test. Other studies report that this is not required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRH agonist stimulation testosterone/oestradiol level can also be used in the assessment of delayed puberty. Discuss with the consultant responsible for the patient about whether they would like this 24-hour blood sample taken.

Use and interpretation of GnRH stimulation test in infants and pre-school aged children

Use of the GnRH stimulation test in young children to establish a diagnosis of CPP has its limitations with respect to the interpretation of results. A peak LH of >5.0 IU/L is commonly used as the diagnostic cut-off for CPP. However, in infants and pre-school aged children, this peak LH cut-off level for central precocious puberty is likely too low.

In a Danish study of 48 healthy girls, <6 years of age, assessed clinically to be pre-pubertal, the following LH and FSH responses, measured on the Roche Cobas e601 platform, were achieved at 30 minutes post Gonadorelin IV injection (0.1mg/m^2 body surface area, maximum dose 0.1 mg):

Table 3 LH and FSH responses at 30 minutes post IV Gonadorelin (Vestergaard ET et al. 2017)

	Age group (years)					
	0–1	**1–2**	**2–3**	**3–4**	**4–5**	**5–6**
Stimulated LH (IU/L) Median (minimum, maximum)	7.57 (5.63–7.66)	4.86 (2.38–8.00)	4.31 (2.84–9.96)	2.19 (1.15–3.92)	3.74 (1.63–5.47)	2.61 (0.87–3.46)
Stimulated FSH (IU/L) Median (minimum, maximum)	26.56 (22.82–40.39)	20.51 (16.62–29.43)	20.14 (9.11–36.15)	12.15 (7.94–19.00)	17.22 (10.40–20.69)	11.53 (6.81–26.95)
Stimulated LH/FSH ratio Median (minimum, maximum)	0.21 (0.19–0.33)	0.25 (0.11–0.29)	0.21 (0.14–0.37)	0.16 (0.06–0.37)	0.26 (0.09–0.43)	0.19 (0.07–0.39)

Notes. FSH, follicle-stimulating hormone; LH, luteinising hormone.

During infancy, usually 1–6 months of age, there is transient activation of the HPG axis, termed ‘mini-puberty of infancy’. Performing a GnRH stimulation test during the mini-puberty of infancy will generate a positive result.

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Yin L, Tang Y, Chen X, Sun Y. Measurement differences between two immunoassay systems for LH and FSH: a comparison of Roche Cobas e601 vs. Abbott Architect i2000sr. *Clin Lab.* 2018;64(3):295–301

GONADOTROPHIN RELEASING HORMONE (GnRH) STIMULATION TEST for the assessment of delayed puberty (New Zealand-specific protocol)

Indication:

Investigation of delayed activation of the hypothalamic-pituitary-gonadal (HPG) axis – constitutional delay vs hypogonadotropic hypogonadism (HH).

Rationale:

Gonadotrophin-releasing hormone (GnRH), secreted by the hypothalamus, stimulates the release of the gonadotropins – luteinising hormone (LH) and follicle-stimulating hormone (FSH) – from the anterior pituitary gland. The pattern of gonadotropin release following stimulation using a GnRH agonist is used to assess the activation and function of the HPG axis.

There is limited evidence of the efficacy of the GnRH stimulation test to distinguish delayed puberty from HH. However, Buserelin has been specifically investigated for this purpose in a single study conducted in New Zealand. The diagnostic utility of anti-Müllerian hormone (AMH) and inhibin B is becoming more recognised, although this has not been completely elucidated.

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs.

Contraindications:

Pregnancy (relative contraindication).

Certain drugs which interfere with GnRH stimulation (it is recommended that potential interactions with drug concomitantly taken by the patient be investigated).

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and intravenous (IV) cannulation skills.

Formulation and Dose:

Table 1 Buserelin formulation and dose

Formulation	Dose	Route
New Zealand		
Buserelin acetate (Suprefact) (1 mg/mL)	100 micrograms	Subcutaneous

Adverse reactions:

Significant adverse reactions have not been encountered. Occasionally, patients may experience nausea, headache, and abdominal pain.

Preparation:

The GnRH stimulation test can be used in combination with other stimulation tests as part of the assessment of pituitary function. When combined with a growth hormone stimulation test, sex-steroid priming is not necessary.

This test can be performed at any time of the day. The patient does not need to be fasting.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – buserelin acetate

Observations:

Observe the temperature, blood pressure (BP), and heart rate (HR) at baseline and then hourly throughout the test, unless stipulated otherwise by the consultant or local policy.

Method:

1. Weigh the patient and take baseline observations.
2. Insert the IV cannula and take baseline (pre-stimulation) bloods samples.
3. Administer the buserelin acetate (dose/route as per table above).
4. Blood sampling as below. If performed as part of a combined pituitary test, see the combined protocol
5. Remove the IV cannula once testing is complete.

Note:

If IV cannulation is not feasible, then bloods can be collected via venepuncture or finger prick/heel prick.

Discharge:

Once the test is complete, ensure the patient meets the discharge criteria as per your local unit.

Sample collection:

Table 2 Buserelin administration and sample collection summary

Drug Administered:		**Dose Administered:**		**Time Administered:**
Actual time bloods taken:	Baseline	Administer triptorelin buserelin	Time after buserelin administration	
Test	-1 Min		4 hours	
LH	✓		✓	
FSH	✓		✓	
Testosterone (males) Estradiol (females)**	✓		✓	
Blood Tubes / Minimum Blood Volume*	SST 2 mL		SST 2 mL	

Notes. FSH, follicle-stimulating hormone; LH, luteinising hormone; SST, serum separator tube; *See Notes section below (regarding the timing of post-GnRH agonist stimulation blood samples); **It is preferable to use a liquid chromatography-tandem mass spectrometry method for oestradiol measurement. This can be ordered via sendaway testing if not available in the local laboratory.

Interpretation:

Based on the levels at 4 hours:

LH and FSH peak post-GnRH agonist <5.0 IU/L – likely HH (LH-preferred)

LH and FSH peak post-GnRH agonist >10 IU/L – normal HPG axis

LH and FSH peak post-GnRH agonist 5–10 IU/L – equivocal but probably normal HPG axis

Notes:

Blood tubes/minimum blood volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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HUMAN CHORIONIC GONADOTROPHIN (hCG) STIMULATION TEST

Indications:

To assess for the presence of functional testicular tissue (that is, functional Leydig cells). For example: in genetic males with ambiguous genitalia, bilateral undescended testes, anorchia (vanishing testes), suspected primary hypogonadism, or following testicular torsion or bilateral orchidopexy.

To assess for testosterone biosynthetic defects or inborn errors of steroidogenesis. For example: 5-alpha reductase deficiency, 17-beta hydroxysteroid dehydrogenase deficiency.

To differentiate between hypogonadotropic hypogonadism (HH) and constitutional delay of growth and puberty.

Please note: There have been numerous studies investigating the relative usefulness of the human chorionic gonadotrophin (hCG) stimulation test in the differentiation of HH and constitutional delay in growth and puberty with variable conclusions. No discriminatory cut-offs are available for this protocol.

Rationale:

Luteinising hormone (LH) is a gonadotropin from the anterior pituitary gland which stimulates Leydig cells in testicular tissue to secrete testosterone. hCG is a polypeptide hormone which shares a common alpha subunit with LH. hCG is, therefore, able to act on the LH receptor of Leydig cells to induce an increase in testosterone biosynthesis and secretion which can be measured within several days of administration. Children aged 6 months to 8 years have a quiescent hypothalamic-pituitary-gonadal (HPG) axis, and therefore gonadal (testicular) function can only be assessed by Leydig cell stimulation using hCG.

Contraindications:

No contraindications in children.

Certain drugs which interfere with GnRH stimulation (it is recommended that potential interactions with drug concomitantly taken by the patient be investigated).

Formulation:

Table 1 Summary of the recombinant human chorionic gonadotrophin (r-hCG), Ovidrel

Product	**Ovidrel** 250 microgram/0.5 mL solution in pre-filled pen Derived from genetically engineered Chinese hamster ovary cells For doses <250 micrograms, the dose can be extracted from the cartridge with a needle
Active ingredient	Choriogonadotropin alfa
Excipients	Mannitol, methionine, poloxamer, monobasic sodium phosphate monohydrate, dibasic sodium phosphate dihydrate, sodium hydroxide, phosphoric acid, water

Dose:

Table 2 Single-dose protocol for Ovidrel

Age	Dose	Route
<2 years old	125 micrograms	Subcutaneous
≥2 years old	250 micrograms	Subcutaneous

Notes: formulations of urinary-derived hCG (uhCG), administered intramuscularly, have been used in many hCG stimulation protocols. However, uhCG is no longer available in Australia and New Zealand and r-hCG, administered subcutaneously, is now used as the stimulant in this test; 250 microgram r-hCG = 6,500 IU uhCG (1 mcg = 26 IU)

There are many different published hCG stimulation test protocols. Please discuss with the consultant to ensure that any desired modifications to hCG administration or sample collections are included.

Adverse reactions:

Local reaction at injection site (irritation, pain, erythema), gastrointestinal upset, and/or headache.

Other side effects relate to prolonged and high-dose administration only.

Preparation:

This test can be performed at any time of day. The patient does not need to be fasting.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

If a GnRH stimulation test is also planned

If the GnRH stimulation test plus hCG stimulation test are being done on the SAME DAY:

- Collect baseline blood samples for BOTH TESTS prior to the administration of GnRH or hCG
- Then, perform the GnRH stimulation test first (this is because hCG has a long half-life and can contaminate the GnRH stimulation test results)

If the GnRH stimulation test is being done AFTER the hCG stimulation test:

- It must be done ≥6 weeks later

Equipment:

Equipment/material required for blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant - Ovidrel pre-filled pen

Observations:

No specific observations are required.

Method:

1. Collect baseline (pre-hCG) bloods
2. Administer hCG as per the dose table above
3. Make arrangements for the post-hCG blood sample to be collected at the appropriate time.

Discharge:

Once the test is complete, ensure the patient meets the discharge criteria as per your local unit. Make sure arrangements have been made for the post-hCG blood test to be conducted.

Sample collection:

Table 3 hCG (Ovidrel) administration and sample collection summary

Drug Administered:	**Dose Administered:**		**Time Administered:**
Actual time & date bloods taken:	Baseline (pre-hCG)	Administer hCG	Post-hCG
Test	-1 Min		7 days
Testosterone	✓		✓
Dihydrotestosterone	✓		✓
Other tests. For example: androstenedione, LH, FSH, DHEAS*, SHBG, AMH, and inhibin B, as per consultant responsible for patient	+/-		+/-
Sample Tubes / Minimum Blood Volume			

Notes. AMH, anti-Müllerian hormone; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; LH, luteinising hormone; SHBG, sex hormone binding globulin; *DHEAS should be measured via liquid chromatography tandem mass spectrometry (LC-MS/MS) – this is not available in all laboratories but can be ordered via sendaway testing.

Interpretation:

Table 4 Testosterone and DHT/T cut-off values with rhCG as stimulant

Stimulation test	Sample time post-hCG	Assay	Cut-off	Interpretation
FOR INDICATION 1				
Single dose r-hCG	7 days after injection	Chemiluminescent Immunoassay (CLIA)	Testosterone <3.7 nmol/L	Suggests no functional testicular tissue (Leydig cells) present + need for testosterone therapy
Single dose r-hCG	7 days after injection	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Testosterone <3.1 nmol/L	
FOR INDICATION 2				
5α-reductase-2 deficiency		LC-MS/MS	T/DHT ratio >10*	Suggestive of 5α-reductase-2 deficiency; warrants genetic test
Note: published ratios have been reported to be between 8 and 10. T/DHT ratio based on Bertelloni et al 2018)				

Notes. CLIA, chemiluminescent immunoassay; hCG, human chorionic gonadotropin; LC-MS/MS, liquid chromatography tandem mass spectrometry; *Ratio refers to both mass units and SI units as the conversion factor for both testosterone and dihydrotestosterone are the same; Published ratios have been reported to be between 8 and 10. The plasma T: dihydrotestosterone (T/DHT) ratio is based on Bertelloni et al. (2018); Strong consideration should be given to genetic testing as ratios vary depending on the protocol used and the method for testosterone and dihydrotestosterone measurement.

This protocol has been published by Oliveria et al. (2022) with the advantage of using rhCG and liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS is more reliable than an immunoassay in assessing 5 alpha reducatase, as immunoassays tend to overestimate testosterone and underestimate DHT.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Other notes

The GnRH agonist stimulation test is more commonly used to assess for HH.

Between 1–6 months of age, the HPG axis is transiently active (mini-puberty of infancy). Random testosterone, LH, and FSH levels taken during this time may provide the information required without the need for an hCG stimulation test.

While hCG stimulates ovarian estrogen and progesterone secretion, it is not employed as a diagnostic test in females.

Whilst a single-dose hCG stimulation regimen may exclude 17β-hydroxysteroid dehydrogenase-3 and 5α-reductase deficiencies, some boys with cryptorchidism may require more prolonged stimulation to assess androgen production and sensitivity.

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Section III

Assessment of disorders of glucocorticoid production

SHORT SYNACTHEN (ACTH) STIMULATION TEST (SST)

Indications:

To assess the response of the adrenal cortex to stimulation from adrenocorticotropic hormone (ACTH) in suspected adrenocortical insufficiency from primary adrenal disease or tertiary/secondary adrenal insufficiency (corticotropin-releasing hormone [CRH]/ACTH deficiency).

Rationale:

ACTH is the primary regulator of glucocorticoid production and also plays a role in adrenal androgen production. Synacthen (tetracosactide), a synthetic form of ACTH, is used to assess the stimulated cortisol response of the adrenal cortex and is valuable in diagnosing suspected primary adrenal insufficiency. The test is also useful in suspected tertiary or secondary (hypothalamic-pituitary) deficiencies, as CRH/ACTH deficiency results in atrophy of the adrenal cortex with a subsequent inability to produce adequate cortisol levels. However, in this setting, the test should not be performed within 6 weeks of the hypothalamic/pituitary insult (for example, pituitary surgery) as atrophy of the adrenal cortex is an evolving process, and within this timeframe the adrenal cortex will still likely be able to produce an adequate cortisol response to Synacthen (tetracosactide) which can be falsely reassuring.

The use of the low-dose (1µg) synacthen test for secondary adrenal insufficiency has been advocated by some clinicians, but this is contentious.

The serum cortisol can be low at the time of hypoglycaemia in neonates with hyperinsulinaemic hypoglycaemia (HH) and, therefore, should be interpreted with caution prior to proceeding with the ACTH stimulation test (synacthen test) in confirmed HH neonates.

Contraindications:

Known hypersensitivity to ACTH.

Ongoing treatment with Synacthen only.

Current treatment with supraphysiological doses of glucocorticoids.

Note: It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Expertise level:

The administration of Synacthen must be by a medical officer and in a clinical area with a full resuscitation facility due to the small but well described risk of hypersensitivity/anaphylaxis. Paediatric clinical nurses may give Synacthen in specialised paediatric units (according to local protocols).

Formulation:

Tetracosactide (Synacthen, solution for injection) 250 mcg in 1 mL. This is a synthetic polypeptide consisting of the first 24 amino acids of the ACTH molecule.

Dose:

Both 250 mcg/m2 and 36 ug/kg are well-accepted dose calculations for the paediatric short Synacthen test. There have been no head-to-head comparisons of the sensitivity of these dosing regimens. The 36 ug/kg dose generates a much higher dose compared to the body surface area (BSA) calculations. Most protocols use 36 mcg/kg in the neonatal period and then an age-specific cut-off for the 125-mcg and 250-mcg doses. Regardless, the doses given are supraphysiological.

Table 1 Standard dose Synacthen test

Age	Dose	Route
0–6 months	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
6 months – 2 years	125 micrograms	Intravenous
Over 2 years	250 micrograms	Intravenous

Adverse reactions:

Hypersensitivity or anaphylactic reactions are rare.

Patients may experience dizziness and nausea.

Preparation:

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed first. For individuals on physiological or sub-physiological glucocorticoid doses, or short courses of supraphysiological doses of glucocorticoids, withhold glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol immunoassay. This is not an issue with the LC-MS/MS method).

The test can be performed any time of the day but preferably before 0900 am in order to appropriately assess basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the short Synacthen test), then the short Synacthen test can be performed at any time of day, as peak cortisol level following ACTH (synacthen) stimulation will still be measurable.

Fasting is not required.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – Synacthen (tetracosactide)

Observations:

Observe the baseline blood pressure (BP), heart rate (HR), and respiratory rate (RR) and hourly thereafter during the test, unless stipulated otherwise by the consultant or local policy.

Method:

1. Document the patient's medication(s) – name of medication, dose, route of administration, time of last dose. Include any glucocorticoids (oral, topical, inhaled, intranasal) or oestrogen therapy.
2. Weigh the patient and take baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer Synacthen (dose and route as per table above).
5. Undertake blood sampling at timepoints, as outlined in table below.
6. If IV access is not available, collect blood samples via finger prick, heel prick, or venipuncture.

Sample collection:

Table 2 ACTH (Synacthen) administration and sample collection summary

Drug Administered:	**Dose Administered:**	**Time Administered:**

Actual time bloods taken:	**Baseline (pre-Synacthen)**	**Administer Synacthen**	**Minutes post Synacthen**	
Test	-1 Min		30 Min	60 Min
Cortisol	✓		✓	✓
ACTH	✓			
Other tests e.g. adrenal androgens as per requesting clinician	**+/-**		+/-	+/-
Blood Tubes / Minimum Blood Volume	SST 1.5 mL EDTA 1 mL (to lab ASAP on cold pack)		SST 1.5 mL	SST 1.5 mL

Notes. ACTH, adrenocorticotropic hormone; ASAP, as soon as possible; EDTA, ethylenediaminetetraacetic acid; SST, serum separator tube

Interpretation:

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in the inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off.

No definitive studies have been performed in the paediatric population to determine cortisol response in healthy children using mass spectrometry-based methods.

The table below describes the minimum cortisol levels achieved in healthy adults post IV Synacthen at 30 minutes for gas chromatography-mass spectrometry (GC-MS) and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than the 30-minute levels.

Table 3 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 and 60 minutes post IV Synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Exercise caution in the interpretation of the cortisol response in patients on oestrogen therapy, such as the oral contraceptive pill (OCP), as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

Notes:

Blood tubes/minimum blood volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Intravenous access

If IV access is not obtainable, administer Synacthen intramuscularly and collect pre- and/or post-Synacthen blood samples via finger prick, heel prick, or venipuncture. Intramuscular Synacthen can only be administered when staff capable of management of anaphylaxis and paediatric resuscitation are available.

Neonates

In neonates <6 months, initial sub-optimal cortisol responses (measured by the Roche GEN I assay on the Cobas e602 analyser) to Synacthen stimulation (defined as <550 nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age.

Timing of SST post-neurosurgery

A Synacthen Stimulation Test should not be performed within 6 weeks of hypothalamic/pituitary insult (for example, pituitary surgery).

A low early-morning (basal) cortisol level during this time can suggest that ACTH deficiency (secondary adrenal insufficiency) is likely. Until the ACTH status of patients at risk of ACTH deficiency is known, an adrenal crisis plan should be in place detailing stress steroid cover during times of illness, further surgery, or other stressors.

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SHORT SYNACTHEN (ACTH) STIMULATION TEST SST
for the diagnosis of congenital adrenal hyperplasia (CAH)

Indications:

For the diagnosis of congenital adrenal hyperplasia (CAH) secondary to 21-hydroxylase deficiency (or a rarer form of CAH) and the assessment of the need for glucocorticoid replacement.

Rationale:

ACTH is the primary regulator of glucocorticoid production and also plays a role in adrenal androgen production. Synacthen (tetracosactide), a synthetic form of ACTH, is used to evaluate the secretion of cortisol, 17-hydroxyprogesterone (17-OHP), and other androgens by the adrenal cortex. In patients with CAH (a group of inherited disorders of adrenal steroidogenesis), there may be inadequate cortisol production. The commonest cause of CAH is due to 21-hydroxylase deficiency which results in the accumulation of 17-OHP, the precursor steroid proximal to the defective enzyme.

Contraindications:

Known hypersensitivity to ACTH.

Ongoing treatment with Synacthen only.

Current treatment with supraphysiological doses of glucocorticoids.

Note: It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Expertise level:

Anaphylaxis to Synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation:

Tetracosactide (Synacthen) 250 mcg in 1 mL. This is a synthetic polypeptide consisting of the first 24 amino acids of the ACTH molecule.

Dose:

Both 250 mcg/m2 and 36 ug/kg are well-accepted dose calculations for the paediatric short Synacthen stimulation test (SST). There have been no head-to-head comparisons of the sensitivity of these dosing regimens. The 36-ug/kg dose generates a much higher dose compared to the body surface area (BSA) calculations. Most protocols use 36 mcg/kg in the neonatal period and then an age-specific cut-off for the 125-mcg and 250-mcg doses. Regardless, the doses given are supraphysiological.

Table 1 Standard dose Synacthen test

Age	Dose	Route
0–6 months	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
6 months – 2 years	125 micrograms	Intravenous
Over 2 years	250 micrograms	Intravenous

Adverse reactions:

Hypersensitivity or anaphylactic reactions are rare. Patients may experience dizziness and nausea.

Preparation:

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning procedure should be performed first. For individuals on physiological or sub-physiological glucocorticoid doses, or short courses of supraphysiological doses of glucocorticoids, withhold glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

The test can be performed any time of the day but preferably before 0900 am in order to appropriately assess basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the short Synacthen test), then the short Synacthen test can be performed at any time of day, as the peak cortisol level following ACTH (Synacthen) stimulation will still be able to be measured.

Fasting is not required.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – Synacthen (tetracosactide)

Observations:

Observe the baseline blood pressure (BP), heart rate (HR), and respiratory rate (RR) and hourly thereafter during the test unless stipulated otherwise by the consultant or local policy.

Method:

1. Document the patient's medication(s) – name of medication, dose, route of administration, time of last dose. Include any glucocorticoids (oral, topical, inhaled, intranasal) or oestrogen therapy the patient is on.
2. Weigh the patient and take baseline observations.
3. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer the Synacthen (dose and route as per table above).
5. Undertake blood sampling at timepoints,as outlined in the table below.

Sample collection:

Table 2 ACTH (Synacthen) administration and sample collection summary

Drug Administered:	**Dose Administered:**	**Time Administered:**

Actual time bloods taken:	**Baseline (pre-Synacthen)**	**Administer Synacthen**	**Minutes post Synacthen**	
Test	-1 Min		30 Min	60 Min
Cortisol	✓		✓	✓
17-hydroxyprogesterone	✓		✓	✓
ACTH	✓			
Other tests, for example, other adrenal androgens as per requesting clinician	**+/-**		**+/-**	**+/-**
Blood Tubes / Minimum Blood Volume*	SST 1.5 mL EDTA 1 mL (to lab ASAP on cold pack)		SST 1.5 mL	SST 1.5 mL

Notes. ACTH, adrenocorticotropic hormone; ASAP, as soon as possible; EDTA, ethylenediaminetetraacetic acid; SST, serum separator tube

Interpretation:

17-OHP levels

Unstimulated 17-OHP levels:

Table 3 Suggested LC-MS/MS cut-off thresholds to exclude CAH

	Unstimulated 17-OHP level
Children	<2.5 nmol/L
Adults	<6 nmol/L

Note. It is important to take the 17OHP sample early in the morning and in the follicular phase in menstruating women.

Stimulated 17-OHP levels:

Table 4 Suggested cut-off thresholds in a short Synacthen test

	Stimulated 17-OHP level at 60 minutes		**CYP21A2 gene status**	**Comment**
	RIA	**LC-MS/MS**		
Normal response	<30nmol/L	<9 nmol/L	No mutation or heterozygous	Phenotype not due to non-classical CAH
Equivocal response	30–43 nmol/L	9–30 nmol/L	Heterozygous or homozygous for two mild mutations (non-classical CAH)	Consider CYP21A2 genotype analysis
Abnormal response	≥43nmol/L	>30nmol/L	Homozygous	Consistent with CAH secondary to 21-hydroxylase deficiency
It is recommended that 17-OHP levels be measured via LC-MS/MS as it has superior specificity.				

Notes. CAH, congenital adrenal hyperplasia; 17-OHP, 17-hydroxyprogesterone; LC-MS/MS, liquid chromatography tandem mass spectrometry; It is recommended that 17-OHP levels be measured via LC-MS/MS as it has superior specificity.

17-OHP cut-offs for the diagnosis of CAH secondary to 21-hydroxylase deficiency have been established using radioimmunoassay (RIA), which is susceptible to inaccuracies associated with cross-reactivity. Limited studies have been published using LC-MS/MS methods.

Carriers for 21-hydroxylase deficiency can produce variable peak 17-OHP levels in the SST, ranging from normal values to 30 nmol/L. This upper value is considered by many investigators as the lower limit for the diagnosis of the non-classical form of CAH.

Cortisol level

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in the inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off. No definitive studies have been performed in the paediatric population to determine cortisol response in healthy children using mass spectrometry-based methods. The table below describes the minimum cortisol level achieved in healthy adults post IV synacthen at 30 minutes for gas chromatography-mass spectrometry (GC-MS)

and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than the 30-minute levels.

Table 5 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 and 60 minutes post IV synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Exercise caution in the interpretation of cortisol response in patients on estrogen therapy such as the oral contraceptive pill (OCP) as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

Additional test

21-deoxycortisol is a steroid intermediate measured by LC-MS/MS which has been found to be a more specific marker for 21-hydroxylase deficiency – especially in the area of newborn screening where prematurity and illness is associated with higher levels of 17-OHP. It has recently been investigated in the SST for identifying carriers of CYP21A2 mutations (HZ) and those with non-classical forms (NC). In this study (Costa-Barbosa FA et al. 2021), 21-deoxycortisol and 17-OHP cutoffs by LC-MS/MS (1.73 nmol/L and 9.38 nmol/L, respectively) correctly recognised 82.5% HZ plus NC, but the combined precursor-to-product ratio [(21-deoxycortisol + 17-hydroxyprogesteron)/cortisol (x10^3)] cutoff of 12 (all in ng/dL) was superior, identifying 92.3% HZ plus NC.

Note: mass unit to SI units (nmol/L) are 21-deoxcortisol (ng/dL multiply by 0.0289), 17-OHP (ng/dL multiply by 0.030), and cortisol (ug/L multiply by 27.6).

Notes:

Blood tubes / minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Intravenous access note

If intravenous access is not obtainable, administer Synacthen intramuscularly and collect pre- and/or post-synacthen blood samples via finger prick, heel prick, or venepuncture. Intramuscular Synacthen can only be administered when staff capable of the management of anaphylaxis and paediatric resuscitation are available.

Neonates note

In neonates <6 months, initial sub-optimal cortisol response (measured on the Roche GEN I assay on the Cobas e602 analyser) tosSynacthen stimulation (defined as <550nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age.

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DEXAMETHASONE SUPPRESSION TEST (DST)
Protocol for overnight low-dose DST

Indications:

To assess for the presence of hypercortisolism

Rationale:

Under normal physiological conditions, the hypothalamic-pituitary-adrenal (HPA) axis involves several steps. Corticotropin-releasing hormone (CRH) secreted from the hypothalamus stimulates adrenocorticotropic hormone (ACTH) production in the anterior pituitary. ACTH acts on the adrenal cortex leading to the production of cortisol. A rise in cortisol level then provides negative feedback to the hypothalamus and anterior pituitary, to suppress/regulate the ongoing production of CRH and ACTH, respectively.

Dexamethasone is a synthetic glucocorticoid that suppresses the HPA axis when given in supraphysiological doses (through negative feedback). Dexamethasone does not interfere with cortisol immunoassay measurements and is suitable for dynamic testing. This is the rationale for its use at different doses in the initial assessment of Cushing syndrome (overnight *low*-dose DST) and when trying to differentiate Cushing disease (ACTH-producing pituitary tumours) from other causes of Cushing syndrome (overnight *high*-dose DST).

Contraindications:

Severe hypertension.

Uncontrolled diabetes mellitus.

Note: It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Precautions:

Exercise caution in children with diabetes mellitus as hyperglycaemia may result. Blood glucose monitoring should be increased, appropriately.

The child should not be on exogenous glucocorticoids (oral, creams, ointments, inhalers, eyedrops) during the test.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing.

Formulation:

Dexamethasone 0.5 mg tablet, 4 mg tablet

Excipients (0.5 mg tab): lactose monohydrate, magnesium stearate, povidone, wheat starch

Excipients (4 mg tab): lactose monohydrate, magnesium stearate, povidone, maize starch

Dose:

Table 1 Dexamethosone dose and administration for low-dose DST

Overnight LOW Dose Dexamethasone Suppression Test
25 micrograms / kg
Maximum:1 mg per dose
Frequency: single dose
Time: administer dose at 23:00

Adverse reactions:

Most side effects from dexamethasone occur when the patient is on high doses for extended periods of time. The single dose used in the DST is unlikely to cause any adverse reactions. Any symptoms experienced are likely to be mild and transient, for example, raised glucose level, sleep disturbance the night of the test, and/or headache.

Preparation:

This test can either be performed in the outpatient setting or inpatient setting (overnight admission). There will be patient and hospital factors that influence the decision as to whether an inpatient or outpatient DST is more appropriate. Liaise with the patient's consultant regarding this.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood sampling –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes

Observations:

On arrival, observe blood pressure (BP), pulse, weight, and height.

Measure blood glucose levels via a glucometer for each blood sample.

Method:

1. Weigh the patient and take baseline observations.
2. Calculate the dexamethasone dose.
3. Collect the blood sample for cortisol and ACTH at 08:30 on Day 1. Depending on patient factors and whether this test is performed in the inpatient or outpatient setting, an IV cannula may be inserted at this point to use for blood sampling on Day 1 and Day 2. The alternative is to undertake two separate venepuncture blood collections (one on Day 1, one on Day 2).
4. Administer the dexamethasone (as per dose section) at 23:00 on Day 1.

Sample collection:

5. Collect the blood sample for cortisol at 09:00 on Day 2.
6. Remove the IV cannula (if one in situ) following completion of the test.
7. Ensure that follow up arrangements are in place for the patient prior to discharge.

Table 2 Dexamethasone administration and sample collection summary

Drug Administered:		**Dose:**	**Time:**	
Actual Time bloods taken:		**Day 1**		**Day 2**
Sample	Tube Blood Volume	08:00 Day 1	23:00 Administer dexamethasone	08:00 Day 2
Cortisol	SST tube 1.0 mL	✓		✓
Dexamethasone (via LC-MS/MS)	SST tube 1.0 mL			✓
ACTH or other analytes only if specified	EDTA (pink) 1.5 mL (on ice)	✓		✓

Notes. ACTH, adrenocorticotropic hormone; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography tandem mass spectrometry; SST, serum separator tube

Dexamethasone measurement via LC-MS/MS is not available in all laboratories but can be ordered via sendaway testing. Although there is some adult data for a minimum dexamethasone level suggestive of adequate absorption, the dexamethasone measurement should be considered optional.

Discharge:

The child must have eaten and have a normal blood glucose level at time of discharge. All observations should be within normal limits; if abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Interpretation:

Table 3 Interpretation of post-dexamethasone cortisol level

Post-dexamethasone cortisol level	**Interpretation**	**Notes**
<50 nmol/L	Cortisol level appropriately suppressed	Suggests that hypercortisolism (Cushing syndrome) is not present, but a second 'normal' screening test is required before excluding the diagnosis.
>50 nmol/L	Cortisol level not appropriately suppressed	Suggests that hypercortisolism (Cushing syndrome) may be present, and further investigation is required.

There is a paucity of paediatric data about the performance of the 50-nmol/L cut-off, but it is well accepted in published guidelines.

No reliable, specific data exist for the sensitivity and specificity of the overnight low-dose dexamethasone suppression test in the paediatric population. In adults, the sensitivity is 95% and specificity is 80%.

If there is incomplete suppression of serum cortisol to <50nmol/L, consider the 48-hour low dose dexamethasone suppression test (LDDST.)

In a small proportion of children with pituitary corticotroph adenomas, suppression to <50nmol/L can occur.

Note: the overnight LDDST is the one screening test for Cushing syndrome. Other screening tests include the 24-hour urinary-free cortisol and serum/salivary diurnal sampling. At least two screening tests should be performed.

Notes:

Blood tubes/minimum collection volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Dexamethasone levels should be at least 5.6 nmol/L to be confident that there have been no issues related to the administration, absorption or metabolism of dexamethasone contributing to a false positive result.

False positive or false negative results

Causes of false positive results include:

- CYP3A4 inducers that increase dexamethasone metabolism, for example, carbamazepine, phenytoin, rifampicin, St John's wort
- Increased corticosteroid-binding globulin (CBG) concentrations which can increase total cortisol concentrations, for example oral estrogens, oral contraceptive pill (OCP), pregnancy, liver problems (chronic active hepatitis)
- Rapid absorption or malabsorption of dexamethasone, for example diarrhoea, coeliac disease, other causes of increased gut transit time

Causes of false negative results include:

- CYP3A4 inhibitors that decrease dexamethasone metabolism, for example fluoxetine, cimetidine, or diltiazem.
- Decreased corticosteroid-binding globulin (CBG) and albumin concentrations, for example kidney or liver problems, such as nephrotic syndrome.

Investigation options to assess for the presence of hypercortisolism (Cushing syndrome)

It is recommended that at least two methods of testing are done to confirm/exclude the presence of hypercortisolism (Cushing syndrome) before considering whether to proceed with second-line investigations to identify the cause of hypercortisolism (Cushing syndrome).

Options include:

- Overnight LDDST or 48-hour LDDST
- 24-hour urine collection for urinary-free cortisol excretion (2–3 samples over 2–3 days)

- Serial cortisol levels (serum or salivary) at 0900 hrs, 1800 hrs, and midnight, for a circadian rhythm profile (for serum cortisol measurements, an IV cannula should be inserted at least 2 hours prior to the sample collection)
- Late night salivary cortisol level collected between 2300–2400 hrs (2–3 samples over 2–3 nights)

Late night cortisol level

There is paediatric data that show a midnight serum cortisol value of ≥4.4 mcg/dL (≥121 nmol/L) confirms the diagnosis of Cushing syndrome in almost all children, with a sensitivity of 99% and a specificity of 100%.

Each laboratory will have its own assay-specific reference range for late-night salivary cortisol levels.

There is adult data which show that late-night salivary cortisol samples collected at bedtime rather than midnight can reduce the rate of false positive results as the circadian rhythm of the cortisol nadir is tightly entrained to sleep onset.

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DEXAMETHASONE SUPPRESSION TEST (DST)
Protocol for 48-hour low-dose DST (LDDST)

Indications:

To assess for the presence of hypercortisolism.

Used as an initial screening instead of the overnight low-dose dexamethasone suppression test (LDDST) or if there is incomplete cortisol suppression following overnight low-dose dexamethasone suppression.

Rationale:

Under normal physiological conditions, the hypothalamic-pituitary-adrenal (HPA) axis involves several steps. Corticotropin-releasing hormone (CRH) secreted from the hypothalamus stimulates adrenocorticotropic hormone (ACTH) production in the anterior pituitary gland. ACTH acts on the adrenal cortex leading to the production of cortisol. A rise in the cortisol level then provides negative feedback to the hypothalamus and anterior pituitary gland, to suppress/regulate the ongoing production of CRH and ACTH, respectively.

Dexamethasone is a synthetic glucocorticoid that suppresses the HPA axis when given in supraphysiological doses (through negative feedback). Dexamethasone does not interfere with cortisol immunoassay measurements and is suitable for dynamic testing. This is the rationale for its use at different doses in the initial assessment of Cushing syndrome (overnight *low*=dose DST or 48-hour low-dose DST) and when trying to differentiate Cushing disease (ACTH-producing pituitary tumours) from other causes of Cushing syndrome (overnight *high*-dose DST).

Contraindications:

Severe hypertension.

Uncontrolled diabetes mellitus.

Note: It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Precautions:

Exercise caution in children with diabetes mellitus, as hyperglycaemia may result. Blood glucose monitoring should be increased appropriately.

The child should not be on exogenous glucocorticoids (oral, creams, ointments, inhalers, eye drops) during the test.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing.

Formulation:

Dexamethasone 0.5 mg tablet, 4 mg tablet

Excipients (0.5 mg tab): lactose monohydrate, magnesium stearate, povidone, wheat starch

Excipients (4 mg tab): lactose monohydrate, magnesium stearate, povidone, maize starch

Dose:

Table 1 Dexamethasone administration summary for 48-hour LDDST

48-hour LDDST	
Dose	30 mcg/kg/day in 4 divided doses for <30 kg; 2 mg/day in 4 divided doses
Frequency	Every 6 hours
Time	Doses administered at 09:00, 15:00, 21:00 and 03:00 for 48 hours

Adverse reactions:

Most side effects from dexamethasone occur when on high doses for extended periods of time. The dose used in the LDDST is unlikely to cause any adverse reactions. Any symptoms experienced are likely to be mild and transient, for example, raised glucose level, sleep disturbance on the night of the test, and/or headache.

Preparation:

The patient should be admitted on the first day, as samples for ACTH are required to be collected at 09:00 and 24:00. There will be patient and hospital factors that influence the decision as to whether an inpatient or outpatient admission is more appropriate for the remainder of the LDDST. Liaise with the patient's consultant regarding this decision.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood sampling –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes

Observations:

On arrival, observe blood pressure (BP), pulse, weight, and height.

Measure blood glucose levels via glucometer for each blood sample.

Method:

1. Weigh the patient and take baseline observations.
2. Calculate the dexamethasone dose.

3. Insert the IV cannula and collect the blood sample for cortisol and ACTH at 09:00 and 24:00 on Day 1.
4. Administer dexamethasone (as per dose section above) on Days 2 and 3 every 6 hours, at 09:00, 15:00, 21:00, and 03:00.

Sample collection:

1. Collect the blood sample for cortisol and ACTH at 09:00 on Day 4.
2. Remove the IV cannula (if one in situ) following completion of the test.
3. Ensure that follow-up arrangements are in place for the patient prior to discharge.

Table 2 Dexamethasone administration and sample collection summary

Drug Administered:	**Dose:**	**Time:**			
Actual Time bloods taken:		**Day 1**	**Day 2**	**Day 3**	**Day 4**
Sample	Tube Blood Volume	09:00 and 24:00	Administer dexamethasone at 09:00, 15:00, 21:00, and 03:00	Administer dexamethasone at 09:00, 15:00, 21:00, and 03:00	09:00
Cortisol	SST tube 1.0 mL	✓			✓
ACTH or other analytes only if Specified	EDTA (pink) 1.5 mL (on ice)	✓			✓

Notes. ACTH, adrenocorticotropic hormone; EDTA, ethylenediaminetetraacetic acid; SST, serum separator tube

Dexamethasone measurement via LC-MS/MS is not available in all laboratories, but it can be ordered via sendaway testing. Although there is some adult data for a minimum dexamethasone level suggestive of adequate absorption, the dexamethasone measurement should be considered optional.

Discharge:

The child must have eaten and have a normal blood glucose level at the time of discharge. All observations should be within normal limits; if abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Interpretation:

Table 3 Interpretation of post-dexamethasone cortisol level

Post-dexamethasone cortisol level	Interpretation	Notes
<50 nmol/L	Cortisol level appropriately suppressed	Suggests that hypercortisolism (Cushing syndrome) is not present, but a second 'normal' screening test is required before excluding the diagnosis.
>50 nmol/L	Cortisol level not appropriately suppressed	Suggests that hypercortisolism (Cushing syndrome) may be present, and further investigation is required.

Paediatric performance data is available for the LDDST. One study with 38 patients reported a sensitivity of 92% while 2 studies with 20 and 48 children, respectively, both reported a sensitivity of 100%.

Note: Like the overnight LDDST, the 48-hour LDDST is one screening test for Cushing Syndrome. Other screening tests include the 24-hour urinary-free cortisol and the serum/salivary diurnal sampling. At least two screening tests should be performed.

Notes:

Blood tubes/minimum collection volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests. as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

False positive or false negative results

Causes of false positive results include:

- CYP3A4 inducers that increase dexamethasone metabolism, for example, carbamazepine, phenytoin, rifampicin, and St John's wort
- Increased corticosteroid-binding globulin (CBG) concentrations which can increase total cortisol concentrations, for example, due to oral oestrogens, oral contraceptive pill (OCP), pregnancy, and liver problems (chronic active hepatitis)
- Rapid absorption or malabsorption of dexamethasone, for example, due to diarrhoea, coeliac disease, and other causes of increased gut transit time

Causes of false negative results include:

- CYP3A4 inhibitors that decrease dexamethasone metabolism, for example, fluoxetine, cimetidine, and diltiazem
- Decreased CBG and albumin concentrations, for example, due to kidney or liver problems such as nephrotic syndrome

Investigation of options to assess for the presence of hypercortisolism (Cushing syndrome)

It is recommended that at least two methods of testing are undertaken to confirm/exclude the presence of hypercortisolism (Cushing syndrome) before considering whether to proceed with second-line investigations to identify the cause of hypercortisolism (Cushing syndrome).

Options include:

- Overnight LDDST or 48-hour LDDST
- 24-hour urine collection for urinary-free cortisol excretion (2–3 samples over 2–3 days)
- Serial cortisol level measurements (serum or salivary) at 09:00, 18:00, and midnight for the circadian rhythm profile (for the serum cortisol measurements, an IV cannula should be inserted at least 2 hours prior to the sample collection)
- Late-night salivary cortisol level collections between 23:00–24:00 (2–3 samples over 2–3 nights)

Late-night cortisol level

There is paediatric data that show a midnight serum cortisol valueof ≥4.4 mcg/dL (≥121 nmol/L) confirms the diagnosis of Cushing syndrome in almost all children, with a sensitivity of 99% and a specificity of 100%.

Each laboratory will have its own assay-specific reference range for late-night salivary cortisol levels.

There is adult data which show that late-night salivary cortisol samples collected at bedtime rather than midnight can reduce false positive results, as the circadian rhythm of the cortisol nadir is tightly entrained to sleep onset.

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DEXAMETHASONE SUPPRESSION TEST (DST)
Protocol for overnight high-dose DST

Indications:

Once the presence of hypercortisolism (Cushing syndrome) has been confirmed, the high-dose dexamethasone suppression test (DST) is one of the subsequent investigations undertaken to assist with identifying the cause of hypercortisolism (Cushing syndrome). The test is used to distinguish Cushing disease adrenocorticotropic hormone (ACTH)-producing pituitary tumours) from ACTH-independent cortisol-producing adrenal tumours or ectopic ACTH production

Rationale:

Under normal physiological conditions, the hypothalamic-pituitary-adrenal (HPA) axis involves several steps. Corticotropin-releasing hormone (CRH) secreted from the hypothalamus stimulates ACTH production in the anterior pituitary gland. ACTH acts on the adrenal cortex leading to the production of cortisol. A rise in the cortisol level then provides negative feedback to the hypothalamus and anterior pituitary gland, to suppress/regulate the ongoing production of CRH and ACTH, respectively.

Dexamethasone, a synthetic glucocorticoid that doesn't interfere with cortisol assay measurements, is able to suppress this HPA axis through negative feedback when given in supraphysiological doses. This is the rationale for its use at different doses in the initial assessment of Cushing syndrome (overnight *low*-dose DST) and when trying to differentiate Cushing disease (ACTH-producing pituitary tumours) from other causes of Cushing syndrome (overnight *high*-dose DST).

Contraindications:

Severe hypertension.

Uncontrolled diabetes mellitus

Note: It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Precautions:

Execrcise caution in children with diabetes mellitus, as hyperglycaemia may result. Blood glucose monitoring should be increased as appropriate.

The child should not be on exogenous glucocorticoids (oral, creams, ointments, inhalers, eye drops) during the test.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing.

Formulation:

Dexamethasone 0.5 mg tablet, 4 mg tablet

Excipients (0.5 mg tab): lactose monohydrate, magnesium stearate, povidone, wheat starch

Excipients (4 mg tab): lactose monohydrate, magnesium stearate, povidone, maize starch

Dose:

Table 1 Dexamethasone administration summary for high-dose DST

Overnight HIGH Dose DST
120 micrograms / kg
Maximum: 8 mg per dose
Frequency: single dose
Time: administer dose at 23:00

Adverse reactions:

Most side effects from dexamethasone occur when on high doses for extended periods of time. The single dose used in the high-dose DST is unlikely to cause any adverse reactions. Any symptoms experienced are likely to be mild and transient, for example raised glucose level, sleep disturbance on the night of the test, and/or headache.

Preparation:

This test can either be performed in the outpatient setting or inpatient setting (overnight admission). There will be patient and hospital factors that influence the decision as to whether an inpatient or outpatient DST is more appropriate. Liaise with the patient's consultant regarding this.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood sampling –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes

Observations:

On arrival, observe blood pressure (BP), pulse, weight, and height.

Measure blood glucose levels via glucometer for each blood sample.

Method:

1. Weigh and measure the patient and take baseline observations.
2. Calculate the dexamethasone dose.

3. Collect the blood sample for cortisol and ACTH at 08:30 on Day 1. Depending on patient factors and whether this test is performed in the inpatient or outpatient setting, an IV cannula may be inserted at this point to use for blood sampling on Day 1 and Day 2. The alternative is two separate venepuncture blood collections (one on Day 1, one on Day 2).
4. Administer the dexamethasone (as per dose section) at 23:00 on Day 1.
5. Collect the blood sample for cortisol at 09:00 on Day 2.
6. Remove the IV cannula (if one in situ) following completion of the test.
7. Ensure that follow up arrangements are in place for the patient prior to discharge.

Discharge:

The child must have eaten and have a normal blood glucose level at the time of discharge. All observations should be within normal limits; if abnormal repeat, as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 2 Dexamethasone administration and sample collection summary

Drug Administered:		**Dose:**	**Time:**	
Actual Time bloods taken:		**Day 1**		**Day 2**
Sample	Tube Blood Volume	08:00 Day 1	23:00 Administer dexamethasone	08:00 Day 2
Cortisol	SST tube 1.0 mL	✓		✓
Dexamethasone (via LC-MS/MS)	SST tube 1.0 mL			✓
ACTH or other analytes only if specified	EDTA (pink) 1. mL (on ice)	✓		✓

Notes. ACTH, adrenocorticotropic hormone; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography tandem mass spectrometry; SST, serum separator tube

Interpretation:

Table 3 Interpretation of post-dexamethasone cortisol level

Post-dexamethasone cortisol level	Interpretation
Suppressed ≥20% from baseline (pre-dexamethasone) cortisol level	Highly suggestive that the cause of hypercortisolism is Cushing's disease (an ACTH-producing pituitary tumour)
Unsuppressed/suppressed <20% from baseline (pre-dexamethasone) cortisol level	Suggests that the cause of hypercortisolism is due to an ACTH-independent cortisol producing adrenal tumour or ectopic ACTH production

Note. ACTH, adrenocorticotropic hormone

Notes:

Blood tubes/minimum collection volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

False positive or false negative results

Causes of false positive results include:

- CYP3A4 inducers that increase dexamethasone metabolism, for example, carbamazepine, phenytoin, rifampicin, St John's wort
- Increased corticosteroid-binding globulin (CBG) concentrations which can increase total cortisol concentrations, for example, due to oral oestrogens, oral contraceptive pill (OCP), pregnancy, and liver problems (chronic active hepatitis)
- Rapid absorption or malabsorption of dexamethasone, for example, diarrhoea, coeliac disease, and other causes of increased gut transit time

Causes of false negative results can include

- CYP3A4 inhibitors that decrease dexamethasone metabolism, for example fluoxetine, cimetidine, and diltiazem
- Decreased corticosteroid-binding globulin (CBG) and albumin concentrations, for example, due to kidney or liver problems such as nephrotic syndrome

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Section IV

Assessment of disorders of glucocorticoid production

ORAL GLUCOSE TOLERANCE TEST (OGTT)
to assess glucose metabolism

Indications:

1. Diabetes Mellitus: to assess glycaemic response to a glucose load in patients thought to be at risk for diabetes, including early (stage 1 or 2) type 1 diabetes mellitus, type 2 diabetes mellitus, cystic fibrosis-related diabetes (CFRD), or atypical (for example, monogenic) diabetes.
2. Non-diabetes: to assess glucose metabolism in suspected conditions other than diabetes mellitus, such as hypoglycaemia, post-prandial hypoglycaemia, and metabolic conditions. The oral glucose tolerance test (OGTT) may be performed after a routine physiological fast or at the end of a formal fasting study.

Rationale:

1. A standardised oral glucose load is administered to assess the ability of the β-cells to appropriately secrete insulin in order to maintain appropriate plasma glucose levels.
2. To assess glucose metabolism after a glucose load.

Contraindications:

Fasting hyperglycaemia >10 mmol/L on the glucose meter – consider terminating the test.

Overt diabetes (symptomatic, fasting plasma glucose ≥7.0 mmol/L or random plasma glucose ≥11.1 mmol/L on two occasions).

Intercurrent illness, for example infection, as the test is invalid in the presence of intercurrent illness.

Recent surgery or trauma which may impair glucose tolerance.

Drugs which can impair glucose tolerance, for example, beta-blockers, corticosteroids, phenytoin, thiazides, or oestrogens – exercise caution.

Notes:

It is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Stage 3b type 1 diabetes mellitus is a clinical diagnosis in a person who is positive for multiple islet autoantibodies, based on the presence of hyperglycaemia (random plasma glucose ≥11.1mmol/l, fasting glucose ≥7.8 mmol/L) and typical osmotic symptoms (for example, polyuria, polydipsia, weight loss) with or without ketosis. An OGTT should not be used in this scenario as it may cause an insulinopaenic child to become very unwell. An OGTT may, however, be useful in individuals with stage 1 (normal glucose tolerance with 2-hour glucose <7.8 mmol/L) or stage 2 type 1 diabetes (impaired glucose tolerance with 2-hour glucose 7.8–11.0 mmol/L) for staging.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing.

Formulation:

Oral glucose solution (centre-specific formulation).

Dose:

1.75 g/kg body weight of glucose dissolved in water, to a maximum of 75 g (body weight ≥ 43kg), consumed within 5 minutes.

Adverse reactions:

About 15% of patients are unable to tolerate glucose solutions, suffering from nausea and vomiting.

Occasionally, patients experience rebound hypoglycaemia towards the end of the test, exhibiting sweating and pallor.

Preparation:

An unrestricted diet with adequate carbohydrate intake for age (in adults: at least 150g carbohydrates per day) for at least three days before the test. This is because carbohydrate restriction can falsely elevate glucose levels with an OGTT.

Normal physical activity, no intercurrent illness.

The test should be performed in the morning after a 10–16-hour overnight fast or other physiological fast duration as specified by the consultant. The fasting time should be shortened for very young children, with the consultant's advice. The test may also be performed after a formal fasting study.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Equipment:

Equipment/material required for intravenous (IV) cannulation and blood sampling –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- Access to hypoglycaemia treatment supplies (see Notes section below)

Observations:

On arrival, observe the blood pressure (BP0, pulse, weight, and height.

Measure blood glucose levels via a glucometer for each blood sample.

Method:

1. Weigh the patient and take baseline observations.
2. Calculate and measure out the volume of glucose solution to be consumed (if not already pre-prepared).
3. Insert the IV cannula.

4. Collect baseline (pre-stimulation) bloods and measure the glucose level using the bedside/point-of-care glucometer.
5. The glucose drink is to be consumed over **no more** than 5 minutes.
6. Emphasise that the patient is to be resting during the test. Water is permitted.
7. The blood samples are collected at timed intervals, as per the table below. Glucose levels are to be measured using the bedside/point-of-care glucometer at each sampling time point. Blood samples are timed from the moment of the first swallow, which is defined as time 0.
8. The patient is to be fed before discharge. Remove the IV cannula if diet and fluids are tolerated.

Discharge:

The child must have eaten and have a normal blood glucose level at the time of discharge. All observations should be within normal limits; if abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 1 Oral glucose administration and sample collection summary

	Baseline	**Oral glucose load**	**1 hour post glucose**	**2 hours post glucose**
Actual time bloods taken				
Test	**-1 Min**		**60 Min**	**120 Min**
Glucose	✓		✓	✓
Other tests e.g. HbA1c, c-peptide as per consultant responsible for patient. DNA extraction and storage if a genetic cause is suspected. Insulin, β-hydroxybutyrate, and lactate if investigating hypoglycaemia.	+/-		+/-	+/-
Sample tubes / Minimum blood volumes	FlOx 0.5 mL		FlOx 0.5 mL	FlOx 0.5 mL

Notes. DNA, deoxyribonucleic acid; HbA1c, glycosylated haemoglobin; FlOx, fluoride oxalate.

Samples at 30 and 90 minutes are often performed in the investigation of hypoglycaemia and for the investigation of cystic fibrosis-related diabetes where these timepoints may be more informative.

Interpretation:

The following values are for glucose levels performed on venous plasma/serum samples. Glucose levels measured on whole blood using glucose meters, bloods gas analysers, or other point-of-care devices should not be used.

Table 2 Glucose level values performed on venous plasma/serum samples

	Plasma/serum glucose level (mmol/L)		
	Fasting		**2 hours**
Normal	<5.6	and	<7.8
Impaired fasting glucose (IFG)	5.6–6.9	and	<7.8
Impaired glucose tolerance (IGT)	<7.0	and	7.8–11.0
Diabetes mellitus	≥7.0	or	≥11.1

Note. Hypoglycaemia and/or hyperlactataemia may be suggestive of a metabolic disorder. See fasting study interpretation.

Notes:

Glucose solution

Commercial glucose preparations (many containing partially hydrolysed starch) are often used in the OGTT. Potential differences between anhydrous/monohydrate forms of glucose in the OGTT has not been sufficiently elucidated.

Treatment options for rebound hypoglycaemia

Table 3 Treatment options for rebound hypoglycaemia

Formulation	**Dose**	**Route**
Glucose 10% intravenous fluid	2 mL / kg	Intravenous bolus/push
Oral glucose gel	15–30 g	Oral
Oral glucose – juice/soft drink	125–250 mL (15–30g carb)	Oral

Note. These are suggested management options for hypoglycaemia. If your local unit has their own hypoglycaemia management guideline, please refer to this.

Blood tubes / minimum collection volume

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Diagnosing & screening for diabetes

In the absence of unequivocal hyperglycaemia (classic symptoms and random plasma glucose ≥11.1mmol/L), the OGTT should be confirmed by repeat testing. The exception is CFRD, where classical diabetes symptoms are often absent.

The use of HbA1c (≥6.5% or ≥48 mmol/mol) for the diagnosis of diabetes, like the OGTT, has not been specifically validated in children and adolescents, and the diagnostic thresholds are all extrapolated from adult definitions.

Screening for type 1 diabetes is occurring more commonly in clinical practice now than previously. Identifying individuals at risk of type 1 diabetes through targeted screening, and monitoring these individuals for onset of diabetes, can lead to earlier diagnosis with a lower likelihood of diabetic ketoacidosis (DKA) at diagnosis. Earlier detection and identification of high-risk individuals also has the potential to provide greater opportunities for these

individuals to participate in studies aimed at delaying/preventing ongoing beta cell destruction. If an individual is found to have two or more islet autoantibodies positive on screening, an OGTT is recommended to stage disease.

Table 4 Stages of type 1 diabetes mellitus

	Islet autoantibodies*	Blood glucose	Symptoms
Stage 1	≥2 autoantibodies positive	Normoglycaemia	Pre-symptomatic
Stage 2	≥2 autoantibodies positive	Dysglycaemia (IFG and/or IGT)	Pre-symptomatic (usually)
Stage 3	≥2 autoantibodies positive	Hyperglycaemia (blood glucose in diagnostic range for diabetes)	Asymptomatic (stage 3a) or Symptomatic (stage 3b)
Stage 4	Established type 1 diabetes		

Note. *Islet tyrosine phosphatase 2 (IA2) antibodies), anti-glutamic acid decarboxylase (GAD) antibodies, anti-insulin antibodies (IAA), and zinc transporter 8 (ZnT8).

Cystic fibrosis-related diabetes (CFRD)

In individuals with cystic fibrosis, an OGTT is still the recommended screening test for CFRD. However, it is important to note that its capacity to identify pathological blood glucose excursions that would be identified by continuous glucose monitoring (CGM) is poor. CGM has not yet been established for the diagnosis of CFRD.

The onset of CFRD is defined as the first time a person with CF meets the criteria for CFRD, even if glucose tolerance subsequently improves. The International Society for Pediatric and Adolescent Diabetes (ISPAD) 2022 Clinical Practice Consensus Guidelines for making a diagnosis of CFRD include:

a. OGTT 2-hour blood glucose level of ≥11.1 mmol/L when the OGTT is conducted during a period of stable health

b. Fasting blood glucose of ≥7.0 mmol/L or 2-hour post-prandial blood glucose of ≥11.1 mmol/L persisting for more than 48 hours during acute illness

c. Blood glucose of ≥11.1 mmol/L mid- or post-feeds on two separate days in an individual on overnight feeds.

Metabolic disorders

The carnitine profile and a urine metabolic screen should be collected if hypoglycaemia occurs during the OGTT.

Samples at 30 and 90 minutes are likely to be required when investigating hypoglycaemic disorders, as the blood glucose level can rebound quickly.

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FASTING TEST PROTOCOL

Indications:

There are several reasons why a fasting test may be indicated:

1) A diagnostic fast –

   To investigate suspected hypoglycaemia, and elucidate a possible cause

   This protocol has been written as a diagnostic fasting test protocol.

2) A safety fast –

   Following an episode of hypoglycaemia (that may or may not have a known cause), a safety fast may be undertaken during admission to ensure that the infant/child is able to fast for an acceptable length of time without developing hypoglycaemia before they are deemed safe for discharge home

3) A medication efficacy fast or a curative fast –

   These fasting tests are most commonly performed in infants/children with hypoglycaemia secondary to hyperinsulinism. Their purpose is to assess whether:

   a. anti-hypoglycaemic medication is effective; and
   b. there has been suspected resolution of disease, including in those who have undergone 'curative surgery' (partial pancreatectomy for focal disease).

Rationale:

The rationale for a diagnostic fasting test –

When baseline studies and clinical information alone have not been able to confirm either the presence of hypoglycaemia or its cause (if hypoglycaemia has already been confirmed), a monitored fasting study in carefully controlled conditions is required to determine whether hypoglycaemia occurs during the fasting period or not, and if it does occur, to also determine what the cause of the hypoglycaemia is by measurement and analysis of relevant metabolites taken at the time of the hypoglycaemic episode. Fasting studies need to be individually planned according to the patient's age and suspected hypoglycaemic disorder. Note, due to the labour- and resource-intense nature of the study, it is preferrable to avoid a fasting study, if possible, by collecting a 'critical sample' in a child (if safe) who presents with confirmed hypoglycaemia, before treatment is initiated.

Contraindications:

Recent or intercurrent illness.

Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Precautions:

A fasting study carries the potential risk of sudden and severe metabolic decompensation, so it is very important to ensure that any patient undergoing any fasting/metabolic investigation has the test performed in a setting where there is close monitoring throughout the test.

Expertise level:

The minimum requirement is for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an intravenous (IV) cannula. This is a potentially a very hazardous test that requires very close supervision by experienced personnel.

Formulation & Dose:

Table 1 Management options for hypoglycaemia during fasting, following collection of the 'critical sample'

Formulation	**Dose**	**Route**
Glucose 10% intravenous fluid	2 mL/kg	Intravenous bolus / push
Oral glucose gel (children)	15–30 g	Oral
Oral glucose – juice / soft drink	125–250 mL (15–30g carb)	Oral
Oral glucose gel 40% (neonates)	0.5 m /kg (200 mg/kg)	Buccal (massage into the inner cheek of the neonate)

Note, the above are suggested glucose options for the management of hypoglycaemia. If your local unit has their own hypoglycaemia management guideline, please refer to that.

Adverse reactions:

Signs and symptoms of hypoglycaemia include sweating, pallor, hunger, nausea, altered behaviour, altered level of consciousness, and seizures.

Cardiac arrhythmias (in fatty acid oxidation disorders).

Preparation:

Ensure the patient has been well prior to commencing the test.

The patient should ideally be consuming adequate carbohydrate content prior to commencing the test (if feasible) to ensure adequate baseline glycogen stores.

The consultant responsible for the patient must specify the plans for fasting (i.e., fasting commencement time, maximum length of fasting), considering the patient's age, size, and likely risk of developing hypoglycaemia after an estimated length of time fasting. The fasting commencement time should be chosen so that any hypoglycaemia is likely to occur during the day when the ward is fully staffed.

Table 2 Suggested times for commencement of fasting and length of fasting based on age

Age	Fasting commencement time	Suggested maximum length of fast
<6 months	04:00	8 hours
6–8 months	00:00 (midnight)	12 hours
8–12 months	20:00 the night prior	16 hours
1–2 years	20:00 the night prior	18 hours
2–7 years	16:00–18:00 the evening prior	20–24 hours
>7 years	16:00 the afternoon prior	24 hours

Consultation with the metabolic and/or paediatric endocrinology team(s) during the planning stages of the fasting test is recommended. If there is a clinical suspicion of glycogen storage disorder (GSD) type 0 or a gluconeogenic disorder, consultation with a metabolic consultant during the planning phase of the test is highly recommended, as they will likely specify that the collection of glucose and lactate 2 hours after treatment with glucose and food is required.

The consultant responsible for the patient must determine if a glucagon stimulation test or oral glucose tolerance test (OGTT) is indicated at the end of the fasting study (see OGTT protocol).

Equipment:

Equipment/material required for IV cannulation, blood collection, and urine collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- urine bottle
- access to ice (to send samples on ice if required)
- bedside glucometer (able to measure glucose levels and ketone levels)
- Access to rapid accurate blood glucose analysis (blood gas machine, iSTAT machine)
- Immediate access to hypoglycaemia treatment (10% glucose IV solution, oral glucose)

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline. The frequency of blood glucose level measurements will be determined by the consultant but should be performed every 30 minutes once the blood glucose level drops below 4.0 mmol/L. Guidance is provided in the table below.

Method:

1. Ensure the steps in the Preparation section have been taken prior to proceeding with the test. Ensure there is appropriate nursing/medical staffing available for the duration of the test to provide close monitoring and supervision.

2. Weigh the patient and calculate the doses of glucose for management of hypoglycaemia.
3. Insert the IV cannula from the time of the last meal (for short fasts) or from 06:00–08:00 (for longer fasts). In children with a history of severe symptomatic hypoglycaemia, 2 IV lines are suggested (if feasible) so that one line can be used for sampling and the other for administration of emergency resuscitation treatment (please check with the consultant responsible for the patient regarding the number of IV lines that should be sited).
4. Take baseline observations.
5. Ensure that the patient has robust IV access and there is ready access to hypoglycaemia treatment in the clinical area prior to commencing the test.
6. Check the blood glucose level hourly (using a glucometer) while the blood glucose level is above 4.0 mmol/L. Consideration should also be given to measuring ketone levels through a point-of-care glucometer, as this may be of diagnostic value if hypoglycaemia does not occur.
7. If/when the blood glucose level falls to 4.0 mmol/L or lower, increase the frequency of blood glucose monitoring, as per the following table –

Table 3 Frequency of blood glucose monitoring

Blood glucose level on glucometer	**Frequency of testing**	**Confirmation required**	**Confirmation method**
Above 4.0 mmol/L	Every hour	No	N/A
3.6–4.0 mmol/L	Every 30 minutes	No	N/A
2.7–3.5 mmol/L	Every 30 minutes	Yes	Blood gas machine, iSTAT. or send to laboratory for rapid processing (to confirm accuracy of glucometer result)
2.6 mmol/L and below (confirm by blood gas machine or iSTAT) OR Symptomatic OR End of pre-determined fasting period	Termination of study	Yes	Blood gas machine, iSTAT, or send to laboratory for rapid processing (to confirm accuracy of glucometer result) Collect blood and urine samples (see sample collection section)
Special circumstance			

Note. *Special circumstance*: **If hyperinsulinism suspected:** when blood glucose level falls *below 4.0 mmol/L*, the frequency of measuring blood glucose levels should be every 15 minutes (rather than every 30 minutes) – consider checking the bedside ketone level at the same time as every blood glucose check.

8. No food until the test is completed / terminated and critical blood / urine samples have been collected.
9. Water / ice is permitted throughout the test to maintain hydration.

Glucagon stimulation test (if requested)

The referring clinician may request a glucagon stimulation test at the end of the fasting test if the patient's blood glucose level has fallen to 2.6 mmol/L or below. This is to assess the availability of glycogen and whether it is able to be mobilised during hypoglycaemia or following glucagon stimulation (for example, investigation of glycogen storage disorder or congenital hyperinsulinism).

In a normal physiological response to fasting, glycogen stores will be depleted and there will not be a glucose rise in response to glucagon administration. A glucose rise in this context at the end of the fast would be inappropriate. In hyperinsulinism, there is an inappropriate response to glucagon stimulation at the end of a fasting study. The inappropriate level of circulating insulin inhibits glycogenolysis. A diagnostic feature of hyperinsulinism is an inappropriately large glycaemic response to glucagon of >1.7mmol/L (30 mg/dL).

If this is the case:

1. Collect the critical blood/urine samples for a hypoglycaemia screen (as per the table; make sure the glucose level and insulin level are included).
2. Administer glucagon –

    Dose: 30 micrograms / kg (max 1 milligram)
    Route: Slow intravenous push

3. Measure glucose (with bedside glucometer and laboratory) and insulin levels at –

    10 minutes, 20 minutes, and 30 minutes post-glucagon administration

During the glucagon stimulation test:

> If the patient develops symptoms of severe hypoglycaemia (seizure, coma), TERMINATE the test and treat the hypoglycaemia with a bolus of 10% glucose IV 2 mL/kg.

If suspecting GSD type 1, use other methods for diagnosis as glucagon stimulation tests can cause adverse outcomes in this scenario.

Discharge:

The child must have eaten, preferably something containing complex carbohydrates, and not have vomited for at least 1 hour post meal. The child must have normal observations and blood glucose level at the time of discharge. If the child's blood glucose remains <3.0 mmol/L at 20 minutes or the child has worsening symptoms of hypoglycaemia, then appropriate hypoglycaemia should be initiated. The child should stay in the ward for 2–4 hours following completion of the fasting test (or a time determined by the doctor). A review by medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 4 Samples to collect at time of hypoglycaemia

SAMPLES TO COLLECT AT THE TIME OF HYPOGLYCAEMIA **Notify the lab when sending a hypoglycaemia screen**
BLOOD
Glucose*
Blood gas*
Lactate*
Ketones – formal sample sent to lab for beta-hydroxybutyrate* and bedside ketones
Insulin*
C-peptide
Cortisol*
Growth hormone
Free fatty acids (send to lab on ice, must arrive within 30 minutes)
Acylcarnitine profile (lithium heparin tube or one spot on newborn screening card/Guthrie card)*
Ammonia (send to lab on ice, must arrive within 30 minutes)
Cytokine, liver function tests, lipids, urate.
URINE (first urine void post hypoglycaemia)
Metabolic screen (organic acids, amino acids)
Ketones (urine dipstick for acetoacetate)

Notes. *If blood collection is difficult and there is limited blood volume collected, these are the most important tests to prioritise from an endocrinological perspective. In a number of cases, the results from these tests can provide enough information to obtain a diagnosis.

Table 5 Blood and urine samples for normoglycaemia

BLOOD AND URINE SAMPLES THAT CAN BE CONSIDERED WHEN NORMOGLYCAEMIC
Full blood count, cytokine, elevated liver function tests, lipids, urate
Early morning cortisol + ACTH (+/- very long chain fatty acids* if suspect Addison's disease in a boy, to assess for adrenoleukodystrophy). If there is a significantly elevated ACTH even in the presence of a robust cortisol level, a short Synacthen test should be performed (which will demonstrate a blunted cortisol rise).
Lactate, ammonia
Growth hormone stimulation test
DNA – for storage or to send away for a specific gene panel (note, there is usually an associated laboratory cost for DNA extraction and storage, hence this should be discussed with the consultant).

Notes. ACTH, adrenocorticotropic hormone; * Very long chain fatty acids should be not mistaken for free fatty acids.

Interpretation:

General principles

In a normal physiological state in response to fasting –

- Blood glucose levels fall
- Free fatty acid levels rise
- Ketone levels rise
- Insulin secretion becomes suppressed
- Counter-regulatory hormone levels (cortisol, growth hormone, glucagon, adrenaline) should be elevated during hypoglycaemia

There are major metabolic pathways involved in glucose homeostasis, as listed below, and the predominant fuel source changes over time as the body shifts from the absorptive phase to the fasted state –

- Exogenous carbohydrates, Glycogenolysis, Gluconeogenesis, Fatty acid oxidation, Ketogenesis and ketolysis

Differential Diagnoses

The table below outlines some of the differential diagnoses to consider based on the timing of hypoglycaemia in relation to the duration of fasting.

Table 6 Differential diagnoses based on the timing of hypoglycaemia

Duration Of Fasting	Predominant Fuel	Differential Diagnoses
0–2 hours	Exogenous carbohydrates (simple sugars to complex carbohydrates)	Hyperinsulinism Dumping syndrome Malabsorption
2–6 hours	Glycogen (glycogenolysis)	Hyperinsulinism Glycogen storage disorders (GSDs) Glucagon deficiency
6–12 hours	Gluconeogenesis	Hyperinsulinism GSD type 0 Gluconeogenesis disorder Idiopathic ketotic hypoglycaemia Glycogen storage disorders Severe fatty acid oxidation disorders
12–24 hours	Fatty acid oxidation	Hyperinsulinism Fatty acid oxidation disorders (FAODs) Growth hormone deficiency Cortisol deficiency Idiopathic ketotic hypoglycaemia

Hypoglycaemia can also be sub-divided into ketotic hypoglycaemia and non-ketotic hypoglycaemia. However, there are many conditions that can present with either ketosis or hypoketosis that are not included on this list (for example, mitochondrial disorders or congenital disorders of glycosylation). The table below includes some of the differential diagnoses to consider.

Table 7 Ketotic and hypoketotic hypoglycaemia – differential diagnoses

KETOTIC HYPOGLYCAEMIA
Idiopathic • Idiopathic ketotic hypoglycaemia
Endocrine Causes • Adrenal insufficiency (cortisol deficiency) • Growth hormone deficiency • Hypopituitarism (ACTH &/or GH deficiency)
Metabolic Causes • Glycogen storage disorders (GSDs) • Gluconeogenic defects • Ketolytic inborn errors of metabolism (IEMs)
HYPOKETOTIC HYPOGLYCAEMIA
Endocrine Causes • Hyperinsulinism
Metabolic Causes • Fatty acid oxidation disorders (FAODs) • Ketogenesis inborn errors of metabolism • Congenital disorders of glycosylation (CDGs can present in many ways but should be considered in a child with multisystem features and hyperinsulinaemic hypoglycaemia) • GSD-I

Notes. ACTH, adrenocorticotropic hormone; GH, growth hormone; GSD-1, glycogen storage disorder type 1

If you have performed a diagnostic fasting test and are unsure how to interpret the results of the investigations, please discuss with your local paediatric endocrinology and / or metabolic teams.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes, as well as any special collection preparations (for example, collection on ice), are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Treatment of hypoglycaemia

Boluses of 50% glucose are *not* recommended as the solution is hyperosmolar which can damage veins, and a rapid bolus can cause rebound insulin release and potentially recurrent hypoglycaemia.

Hypoglycaemia in infancy

Hypoglycaemic in infancy has a high yield of abnormal metabolic/endocrine investigations.

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Section V

Assessment of disorders of water homeostasis

WATER DEPRIVATION TEST

Indications:

For the investigation of polyuria-polydipsia syndrome and to assist in differentiating between arginine vasopressin (AVP) deficiency, AVP resistance, and primary polydipsia.

Rationale:

The water deprivation test (WDT) is used in polyuria-polydipsia syndrome to distinguish diabetes insipidus (DI) from primary polydipsia. It is an indirect test used to determine whether there is AVP deficiency (cranial/central DI), AVP resistance (nephrogenic DI) or primary polydipsia by assessing what happens to the urine osmolality and plasma osmolality/sodium when fluid intake is restricted.

AVP (also known as anti-diuretic hormone [ADH]) is a principal hormone involved in the regulation of water/fluid balance. It is released from the posterior pituitary gland in response to increasing plasma osmolality and acts on V2 receptors in the kidney. This promotes reabsorption of water via aquaporin channels, which leads to declining urine volumes, increasing urine osmolality, and prevention of further increase in plasma osmolality; thereby, maintaining plasma osmolar homeostasis. If there is AVP deficiency or resistance, this water-balance feedback loop is disrupted with development of polyuria (passage of large volumes of dilute urine) and compensatory polydipsia (increased thirst) in those with an intact thirst mechanism. Polyuria is also a feature of primary polydipsia and can be associated with impaired renal concentrating capacity resulting in low urine osmolality.

After non-AVP related causes have been excluded in children and adolescents with polyuria polydipsia syndrome (PPS), paired morning serum and urinary sodium and osmolality should be measured. If the results are conclusive for AVP deficiency (Na >145 mmol/L with urine osmolality <300 mOsm/kg), no further diagnostic testing is required. However, if the paired samples are inconclusive, assessment of AVP production using the current gold standard, a WDT, is indicated. Of note, alternatives to the WDT with better tolerability and greater sensitivity and specificity are being explored. The glucagon-stimulated copeptin test has shown promising data in adults and is under investigation as a potential replacement for the WDT in children (see Appendix).

Contraindications:

Existing dehydration (hypovolaemia) or electrolyte abnormality, intercurrent illness, renal insufficiency, uncontrolled diabetes mellitus, or uncorrected thyroid or adrenal deficiency.

Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Precautions:

Desmopressin should be used with caution in patients with hypertension or coronary artery disease.

Expertise level:

This is a potentially dangerous test and requires strict supervision to avoid dehydration and electrolyte disturbance and ensure diagnostic samples are collected appropriately.

This procedure should only be performed at a specialist centre with known expertise.

Preparation:

Prior to proceeding with a WDT, if it is unclear from the information available whether polyuria is truly present (rather than urinary frequency), consider a 24–48-hour inpatient admission for strict fluid balance monitoring to objectively measure and document fluid input and output and to confirm whether polyuria is present or not.

For any child with confirmed polyuria, before proceeding with the WDT, undertake measurement of a random, baseline copeptin level. If the copeptin level is elevated (>21.4 pmol/L), a WDT will not be required as this excludes AVP deficiency, and AVP resistance should be considered. Please note, this cut-off should be used with caution in children with renal insufficiency, as clearance of copeptin will be affected.

Exclude other causes of polyuria/polydipsia, such as hyperglycaemia (diabetes mellitus), hypercalcaemia, hypokalaemia, urinary tract infection, chronic renal failure, administration of large volumes of sodium chloride 0.9% (sodium-induced polyuria), and medications (for example, mannitol, diuretics).

Ensure there is adequate replacement of thyroxine and/or cortisol in patients on medication for hypothyroidism and/or hypoadrenalism, respectively.

Discus with the consultant responsible for the patient regarding:

a) Whether the WDT is performed in the inpatient or day unit setting

b) What time of day the WDT will start

c) The maximum length of time the WDT will be conducted

d) Any additional tests that are required before commencing the test (specify which tests, if any, are required on the request form)

Inform your hospital laboratory of the date and time the WDT will be performed so that they can ensure adequate staff are available for the urgent processing and reporting of results of samples collected during the period of testing.

Note – desmopressin route of administration

Oral administration of desmopressin is NOT the preferred route of administration due to unpredictable absorption. If the patient has nasal congestion or it is anticipated that intranasal administration will be challenging, then either the intravenous (IV), intramuscular, or subcutaneous route is recommended.

TAKE CARE: The potency of desmopressin differs depending on the route of administration; that is, different dosing routes are NOT equivalent.

Table 1 A guide for desmopressin potency based on route of administration

Intravenous, intramuscular, subcutaneous	10 x more potent than intranasal
Intranasal	10 x more potent than oral
Oral	Least potent

Note, desmopressin is also available as a sublingual wafer. The potency of a 120-microgram wafer is equivalent to a 200-microgram tablet when it is administered as a whole wafer, sublingually. Dose equivalence when the wafer is divided or the wafer is dissolved in water and administered orally has not been established.

Formulation & Dose:

Table 2 Formulation, dose and route of desmopressin

Formulation	Dose	Route
Desmopressin injection 4 mcg/mL (1 mL ampoule)	0.1 microgram (<2 years) 0.2 microgram (2 to <6 years) 0.3 microgram (6 to <10 years) 0.4 microgram (10 to <12 years) 0.6 microgram (12 to <14 years) 0.8 microgram (14 to <16 years) 1.0 microgram (16 to 18 years)	Intravenous

Note. Doses based on Australian Medicines Handbook Children's Dosing Companion

Adverse reactions:

This is a potentially dangerous test. Excessive water deprivation may cause significant dehydration and electrolyte disturbance (*hyper*natraemia, in particular). Desmopressin administration needs careful supervision to avoid overhydration and electrolyte disturbance (*hypo*natraemia, in particular).

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- urine pan/container/urinal, measuring equipment for urine and urine pottles
- digital weighing scales (to weigh patient)
- desmopressin
- worksheet

Observations:

Blood pressure (BP) and heart rate (HR) hourly.

Blood glucose level with every blood test.

Serial weights/ bloods/urine as per 'Water Deprivation Test – Worksheet for use during testing' (refer below).

Method:

Prior to starting the WDT, liaise with the consultant responsible for the patient and complete the 'Water Deprivation Test – Preparation Worksheet' (refer below).

Close 1:1 supervision is required throughout the WDT to ensure the patient DOES NOT HAVE ACCESS TO ANY WATER/FLUID OR FOOD. This includes supervision in the bathroom to ensure the patient is not seeking water from bathroom taps, a shower, or toilet bowl.

At the time of the WDT, please use the 'Water Deprivation Test – Worksheet for use during testing' (refer below). This provides details on when to collect blood/urine samples and when to perform measurements (urine measurements, weights, pulse, blood pressure). It provides space to record the results of all measurements.

Please refer below to 'Water Deprivation Test – Worksheet for use during testing' for *Termination Criteria*.

IF ANY OF THESE CRITERIA ARE REACHED DURING THE TEST, CONTACT THE DOCTOR URGENTLY (if not already present) to notify them and discuss the next step, for example, administration of desmopressin or cessation of test. DO NOT let the child drink/eat unless the appropriate blood/urine samples have been collected and the doctor has instructed that the period of fasting can cease.

On the day of the WDT (based on commencement time of 08:00):

- Child to empty bladder on waking (this may be at home if coming in on the day of the test).
- Notify laboratory the WDT will be commencing.
- Obtain IV access.
- Weigh the child (document clothing and other items included in weight, for example, IV cannula, arm board).
- Calculate weight for 5% dehydration (that is, 95% of baseline weight)
- 08:00: start of WDT (= Time 0)
- Tale measurements and sample collections as per 'Water Deprivation Test – Worksheet for use during testing'.

Complete the following table:

Table 3 WDT schedule

Location where WDT will take place (circle location)	Inpatient/Day Unit
Date/time that patient should arrive (consider admitting the night prior to test vs coming in on the morning of test)	
Date/time that patient should stop eating food	
Date/time that patient should stop drinking fluids*	
Date/time that WDT will commence (often 08:00)	
Date/time that period of water deprivation should cease (if 'Termination Criteria' are not met prior)**	
Staff to be present for the duration of WDT (circle which staff are required)	Nurse/nurse specialist/nurse practitioner/resident medical officer/consultant

Notes. *The patient's age, degree of polyuria and anticipated rate of dehydration need to be taken into consideration. In young children and/or when rapid dehydration is anticipated, it is recommended that water deprivation is commenced first thing in the morning, for example, at 08:00 following consumption of breakfast (with no more than 100–150 mL of fluid consumed). Where less rapid dehydration is anticipated, for example, where the child usually sleeps through the night without drinking, fluids may be ceased the night before.

**Maximum duration of water deprivation should not exceed 4 hours in a newborn, 8 hours in a 3–12-month-old, or 12 hours in a 1–2-year-old. It is rare for water deprivation to continue >12–16 hours in a child of any age or >18 hours in an adult.

Water Deprivation Test – Worksheet for use during testing

Table 4 WDT – worksheet for use during testing

Date	
Weight (kg)	Baseline = 95% of baseline weight (that is, 5% of dehydrated weight) =
Height (cm)	
Current medication (include doses plus date/time of last dose)	
Desmopressin	Dose given = Time given = Route of administration =

Record sheet: Baseline bloods are to be collected at the commencement of fasting or at the time of cannulation if fasting has already commenced at home.

Table 5 WDT – record sheet for use during testing

Timepoint	Actual Time	Weight (kg)	Pulse (bpm)	BP (mmHg)	Glucose (mmol/L)	Plasma sodium (mmol/L) and osmolality (mOsm/kg)	Urine sodium and osmolality (mOsm/kg)	Urine volume (mL)**	Copeptin (baseline and termination)
0 (baseline)									
+ 1 hr									
+ 2 hrs									
+ 3 hrs									
+ 4 hrs									
+ 5 hrs									
+ 6 hrs									
+ 7 hrs									
+ 8 hrs									
+ 9 hrs									
+ 10 hrs									
+ 11 hrs									
+ 12 hrs									
+ 13 hrs									
+ 14 hrs									
+ 15 hrs									
+ 16 hrs									
Post-desmopressin administration (record desmopressin dose, time, and route of administration in box at top of worksheet)***									
+ 1 hr									
+ 2 hrs									
+ 3 hrs									
+ 4 hrs									

Notes. **Record volume of all urine passed and reserve a 10 mL aliquot of any urine passed between the timepoints to send to lab if no urine passed within 30 minutes of subsequent timepoint.

**If you have access to a refractometer, urine specific gravity can be measured at the bedside at each time point in addition to sending urine samples to the laboratory for urine osmolality. Urine specific gravity results can be recorded to the right of the 'Urine volume' column of the table. As a guide, to work out the urine osmolality from the urine specific gravity, take the last two digits of the urine specific gravity and multiply by 30. For example:

- Urine specific gravity = 1.005, then urine osmolality = 05 x 30 = 150 mOsm/kg
- Urine specific gravity = 1.010, then urine osmolality = 10 x 30 = 300 mOsm/kg
- Urine specific gravity = 1.030, then urine osmolality = 30 x 30 = 900 mOsm/kg

***Prior to administration of desmopressin, ensure that a blood/urine sample has just been collected (to use as the baseline sample for pre/post desmopressin comparison). Desmopressin can then be administered at the dose/route recommended by the treating clinician. The child may also drink up to 200 mL water at this point if they wish, prior to resuming fasting. The response to Desmopressin is then observed over the following 2 – 4 hours (2 hours in infants, 4 hours in children/adults). Collect a urine sample for urine osmolality up to hourly following desmopressin administration (note: if there is a positive response to desmopressin the frequency of voiding could reduce to less than hourly). With each urine sample collected, also collect a simultaneous/paired blood sample (do not collect a blood sample more frequently than every hour).

In addition to the above tests, it is also recommended to measure potassium, urea, and creatinine at each blood sampling timepoint. Other additional tests are at the discretion of the treating clinician.

Termination Criteria:

Once the following termination criteria are met, send urine and bloods at this timepoint for urgent sodium and osmolality testing.

Table 6 WDT termination criteria

Plasma/serum sodium	>147 mmol/L
Plasma osmolality	>300 mOsm/kg
Urine osmolality	>750 mOsm/kg* (or > 500mOsm/kg in infants) OR Consistently <30 mOsm/kg between 3 consecutive samples
Weight	>5% loss from baseline weight Note: ensure that weight is performed on the same scales

Note. *Check with supervising consultant. Local termination criteria may use urine osmolality 800mOsm/kg.

IF ANY OF THESE CRITERIA ARE REACHED DURING THE TEST, CONTACT THE DOCTOR URGENTLY (if not already present) to notify the doctor and discuss the next step, for example, administration of desmopressin or cessation of test. DO NOT let the child drink/eat unless the appropriate blood/urine samples have been collected and the doctor has instructed that the period of fasting can cease.

The copeptin level should be performed if the termination criteria are met (see table above). Hyperosmolality is a stimulus for both copeptin and AVP secretion. AVP and copeptin are both peptides derived from ‘pre-pro-vasopressin' and are secreted in equimolar amounts. AVP is difficult to measure for technical reasons while copeptin is a simple, sensitive, and stable analyte to measure, making it a useful surrogate marker for AVP secretion. The copeptin measurement takes less than 2 hours once on the analyser, but the results may take several days to be available (depending on whether the test is performed onsite or sent away to another laboratory); however, the measurement can still be of great assistance in differentiating between central diabetes insipidus and primary polydipsia. See ‘Interpretation’ section for further details.

Sample collection:

Table 7 Summary of sample collection

Plasma sodium, glucose, potassium, urea, creatinine, osmolality, copeptin	2 mL Plain, PST (with lithium heparin gel), SST, Lithium heparin
Urine osmolality	1–10 mL in the urine collection bottle/tube specific for your laboratory

Notes. PST, pancreastatin; SST, serum separator tube

Interpretation:

Table 8 Interpretation of urine and plasma osmolality

	Urine osmolality (mOsm/kg)		Plasma osmolality post-dehydration (mOsm/kg)
	Post-dehydration	**Post-desmopressin**	
Normal	>750 mOsm/kg (or >500mmol/kg in infants)	Desmopressin administration NOT required	< 300 mOsm/kg
AVP resistance	<300 mOsm/kg	≤10% increment in urine osmolality 2 hours post-DESMOPRESSIN	≥ 300 mOsm/kg
AVP deficiency	<300 mOsm/kg	15–100% increment in urine osmolality 2 hours post-DESMOPRESSIN	≥ 300 mOsm/kg
Partial AVP deficiency	300–750mOsm/kg	15–100% increment in urine osmolality 2 hours post-DESMOPRESSIN	≥ 300 mOsm/kg
Primary polydipsia/ other	Plasma sodium and osmolality are maintained within the normal range. Maximum urine osmolality may not rise above 750 mOsm/kg after water deprivation, as the ability to concentrate urine in primary polydipsia may be impaired in response to excessive habitual drinking. A urine osmolality between 300–750 mOsm/kg may also indicate an unsatisfactory test, partial DI, chronic renal failure or diuretic administration		

Note. AVP, arginine vasopressin.

Copeptin-based diagnosis in polyuria-polydipsia syndrome

Table 9 Summary of copeptin-based diagnosis in polyuria-polydipsia syndrome

Condition under which copeptin level is measured	**Diagnosis**	**Interpretation**	
		Adults	**Children**
Random measurement	AVP resistance	>21.4 pmol/L (100% sensitivity, 100% specificity)	>20 pmol/L
Serum sodium >147 mmol/L or plasma osmolality ≥ 300 mOsm/kg (random or post water deprivation)	AVP deficiency	≤4.9 pmol/L (93% sensitivity, 100% specificity)	<2.2 pmol/L
Serum sodium >147 mmol/L or plasma osmolality ≥300 mOsm/kg (random or post water deprivation)	Primary polydipsia	>4.9 pmol/L (100% sensitivity, 93% specificity)	>5 pmol/L to 20 pmol/L
60 minutes following commencement of arginine infusion during an arginine-stimulated copeptin test	AVP deficiency	≤3.8 pmol/L	
In children, copeptin levels 2.2–5.0 pmol/L may be seen in partial AVP deficiency and primary polydipsia and cannot be differentiated without measurement of plasma and urine osmolality.			

Notes. AVP, arginine vasopressin; Fenske W et al. NEJM 2018; 379:428–9; Tuli G et al. Clin Endo 2018; 88:873–879.

Notes

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

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Section VI

Combined protocols

Combined Pituitary Function Test
GH Stimulation Test (Glucagon and Arginine) and
Short Synacthen Test (Synacthen [ACTH])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Glucagon stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency
Short Synacthen test	To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency

Notes. ACTH, adrenocorticotropic hormone; GH, growth hormone.

Arginine stimulates growth hormone (GH) secretion by (1) stimulation of α-adrenergic receptors and subsequent growth-hormone-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Glucagon stimulates GH secretion by stimulation of α-adrenergic receptors and subsequent GHRH release. It also causes a rise in blood glucose levels and subsequent insulin secretion which indirectly stimulates GH and adrenocorticotropic hormone (ACTH) secretion.

Contraindications:

Severe renal, cardiac, or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce GH and cortisol response).

Failure to eat for 48 hours, or a glycogen storage disorder (GSD), or severe cortisol deficiency. In these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur.

Diabetes (the glucagon stimulation test is unreliable in individuals with diabetes, as this GH 'stimulus' requires endogenous insulin).

Certain drugs, for example, periactin, which interfere with arginine stimulation. Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Known allergic tendencies

Known hypersensitivity to ACTH.

Current treatment with supraphysiological doses of glucocorticoids.

Ongoing treatment with Synacthen only.

Precautions:

Ensure the patient has robust intravenous (IV) access for arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test.

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Anaphylaxis to Synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation and dose:

Table 2 Arginine formulation, dose, and route of delivery

Formulation	**Dose**	**Route**
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Glucagon formulation, dose, and route of delivery

Formulation	Dose	Route
Glucagon hydrochloride (1mg; powder plus diluent)	30 mcg/kg (max 1mg)	Subcutaneous

Table 4 Synacthen formulation, dose and route of delivery

Formulation	Dose		Route
Tetracosactide (Synacthen, solution for injection) 250 mcg in 1 mL	0–6 months old	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
	6 months–2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis – extremely rare.

Hypotension requiring intravenous fluid replacement – rarely observed one hour after the administration of arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests – there have been case reports.

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Glucagon

Transient nausea, flushing, vomiting for 1–2 minutes, abdominal pain/cramps, and feelings of apprehension may occur.

Anaphylaxis is a very rare, but potential, complication.

Note: Glucagon stimulates a 2–3-fold rise in blood glucose level following administration. This is maximal within the first hour. Following this rise in blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Synacthen

Hypersensitivity or anaphylactic reactions – rare.

Dizziness and nausea.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the growth hormone stimulation test (GHST).

In individuals on chronic, supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed before undertaking a short Synacthen test (SST). For individuals on physiological or sub-physiological glucocorticoid doses or short courses of supraphysiological doses of glucocorticoids, withhold the glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

The test can be performed any time of the day but preferably before 09:00 in order to appropriately assess the basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the SST), then the SST can be performed at any time of day as the peak cortisol level following ACTH (Synacthen) stimulation will still be measurable.

In patients who have recently undergone neurosurgery and are at risk of ACTH deficiency (secondary adrenal insufficiency), check with the consultant responsible for the patient about the desired timeframe post-surgery for arranging the SST. Following the loss of endogenous ACTH supply, the adrenal glands will eventually atrophy and no longer be able to produce adequate cortisol levels. However, this process takes time, and in the first approximately 6 weeks after the onset of ACTH deficiency (as a result of neurosurgery), the adrenal glands will still be able to produce an adequate (normal), but falsely reassuring, response to exogenous ACTH (Synacthen) during an SST. A low, early-morning (basal) cortisol level during this time can suggest that ACTH deficiency (secondary adrenal insufficiency) is likely. Until the ACTH status of patients at risk of ACTH deficiency is known, there should be a plan in place for stress steroid cover during times of illness, further surgery, and other stressors.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of estrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, eligibility criteria for growth hormone through Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

Table 5 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20 kg: 1 mg once daily orally Weight >20 kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement – discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, glucagon, synacthen

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test, unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh the patient and take baseline observations.
3. Work out and prescribe the arginine, glucagon, and Synacthen doses.
4. Insert the IV cannula and take the baseline (pre-stimulation) blood samples.
5. Administer the glucagon subcutaneously (dose as per dosing table above).
6. Administer the Synacthen as a push intravenously, followed by a sodium chloride 0.9% flush.
7. Administer the arginine via IV infusion over 30 minutes. The time that the infusion STARTS (not finishes) is Time 180 minutes. Ensure a 10–15 mL flush with sodium chloride 0.9% prior to taking the 210-minute blood sample.
8. Blood sampling at timepoints, as outlined in the table below.
9. Continue the blood sampling at the timepoints as outlined in the table below.

11. Check the blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
12. No food (other than for the treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge; if abnormal, repeat as required. A review by medical or nursing personnel (as per local practice) is to be conducted prior to discharge.

Sample collection:

Table 6 Glucagon and arginine/Synacthen administration, dose and sampling summary

Drug Administered			**Dose Administered**						**Time Administered**				
	Baseline		**Minutes post START of glucagon injection**										
Actual time bloods taken		Administer glucagon						Administer arginine and synacthen					
Test	-1 Min		60 Min	90 Min	120 Min	150 Min	180 Min		210 Min	225 Min	240 Min	255 Min	270 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Cortisol	✓								✓		✓		
ACTH	✓												
Other tests, for example, IGF1, IGFBP3, ACTH, cortisol at baseline, and various timepoints as per requesting clinician	+/-												
Sample Tubes/ Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL						

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

Interpretation:

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the Pharmaceutical Benefits Scheme (PBS) in Australia or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and PHARMAC.

Table 7 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube.

Table 8 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test.

Short Synacthen Test Interpretation

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in the inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off.

No definitive studies have been performed in the paediatric population to determine the cortisol response in healthy children using mass spectrometry-based methods.

The table below describes the minimum cortisol level achieved in healthy adults post IV Synacthen administration at 30 minutes for gas chromatography-mass spectrometry (GC-MS) and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than those recorded at 30 minutes.

Table 9 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects at 30 and 60 minutes post IV Synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. GC-MS, gas chromatography-mass spectrometry; OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Cortisol level in neonates

In neonates <6 months, the initial sub-optimal cortisol responses (measured on Roche GEN I assay on the Cobas e602 analyser) to Synacthen stimulation (defined as <550nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age and have higher 30-minute cortisol responses on initial testing (390 nmol/L vs 181 nmol/L).

SST Interpretation note

Although both 30-minute and 60-minute samples are recommended, the 30-minute cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Exercise caution in the interpretation of the cortisol response in patients on oestrogen therapy, such as the oral contraceptive pill (OCP), as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

REFERENCES

See individual protocols.

Combined Pituitary Function Test
GH Stimulation Test: (Arginine and Clonidine) and
Short Synacthen Test (Synacthen [ACTH])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Clonidine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Short Synacthen test	To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency

Notes. ACTH, adrenocorticotropic hormone; GH, growth hormone

Arginine and clonidine stimulate growth hormone (GH) secretion by (1) stimulation of α-adrenergic receptors and subsequent growth-hormone-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Contraindications:

Severe renal, cardiac, or liver disease.

Sick sinus syndrome, compromised intravascular volume, hypotension, syncope, or autonomic dysfunction.

Children with known congenital/acquired heart disease – exercise caution.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce GH and cortisol response).

Certain drugs, for example, periactin, that interfere with arginine stimulation. Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Known allergic tendencies.

Known hypersensitivity to ACTH.

Current treatment with supraphysiological doses of glucocorticoids.

Ongoing treatment with Synacthen only.

Precautions:

Ensure the patient has robust intravenous (IV) access for arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Anaphylaxis to synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation and dose:

Table 2 Arginine formulation, dose, and route of delivery

Formulation	Dose	Route
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Clonidine formulation, dose, and route of delivery

Formulation	Dose	Route	Notes
Clonidine	100 micrograms/m2 orally (maximum 250 micrograms)	Oral	Calculate dose to nearest half tablet

Notes. Clonidine 100-microgram and 150-microgram tablets are available on the PBS in Australia. Clonidine 25-microgram and 150-microgram tablets are available in New Zealand.

Table 4 Synacthen formulation, dose and route of delivery

Formulation	Dose		Route
Tetracosactide (Synacthen, solution for injection) 250 mcg in 1 mL	0–6 months old	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis – extremely rare.

Hypotension requiring intravenous fluid replacement – rarely observed one hour after the administration of arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests – there have been case reports.

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of the arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Clonidine

Drowsiness 1–3 hours post ingestion, nausea, and vomiting.

Hypotension, postural hypotension – fall in blood pressure by approximately 10 mmHg about 1 hour after ingestion which usually resolves by the end of the test but may last several hours. The effect is prolonged in renal failure. A bolus of 10 mL/kg 0.9% sodium chloride administered over 30 minutes following clonidine administration can minimise the fall in blood pressure.

Synacthen

Hypersensitivity or anaphylactic reactions – rare.

Dizziness and nausea.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the growth hormone stimulation test (GHST).

If on regular antihypertensive medication, please check with the consultant responsible for the patient about withholding this medication prior to the test.

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed before undertaking a short Synacthen test (SST). For individuals on physiological or sub-physiological glucocorticoid doses or short courses of supraphysiological doses of glucocorticoids, withhold the glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

The test can be performed any time of the day but preferably before 09:00 in order to appropriately assess the basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the SST), then the SST can be performed at any time of day as the peak cortisol level following ACTH (synacthen) stimulation will still be measurable.

In patients who have recently undergone neurosurgery and are at risk of ACTH deficiency (secondary adrenal insufficiency), check with the consultant responsible for the patient about the desired timeframe post-surgery for arranging the SST. Following the loss of endogenous ACTH supply, the adrenal glands will eventually atrophy and no longer be able to produce adequate cortisol levels. However, this process takes time, and in the first approximately 6 weeks after the onset of ACTH deficiency (as a result of neurosurgery), the adrenal glands will still be able to produce an adequate (normal), but falsely reassuring, response to exogenous ACTH (Synacthen) during an SST. A low early-morning (basal) cortisol level during this time can suggest that ACTH deficiency (secondary adrenal insufficiency) is likely. Until the ACTH status of patients at risk of ACTH deficiency is known, there should be a plan in place for stress steroid cover during times of illness, further surgery, and other stressors.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Note: children with ADHD may be on clonidine treatment doses of up to 400 mcg daily. The clonidine should be withheld the day before and on the day of the test. Consideration should be given to using an alternative protocol, including either arginine or glucagon.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of estrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

Table 4 Sex-steroid priming options for males and females

Formulation	Dose	Duration
Ethinylestradiol	40 mcg/m2 orally in 2 divided doses per day	In the 2 days before the day of GH stimulation testing
Estradiol valerate	Weight ≤20kg: 1 mg once daily orally Weight >20kg: 2 mg once daily orally	In the 2 days before the day of GH stimulation testing
Estradiol patches	25 mcg/24 hours: one patch for 48 hours	In the 2 days before the day of GH stimulation testing

Note. GH, growth hormone; Estradiol side effects can include moderate and transient breast enlargement – discontinue if nausea and vomiting occur.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, clonidine, Synacthen

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test, unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps from the Preparation section have been taken prior to proceeding with the test. Ideally, perform the test first thing in the morning following an overnight fast (see the fasting protocol for age-based maximum fasting durations). However, a minimum fasting time of only 2 hours is required, and this shorter fasting time should be applied to infants and young children.
2. Weigh the patient, calculate the arginine, clonidine, and Synacthen doses, and take baseline observations.
3. Insert the IV cannula and take the baseline (pre-stimulation) blood samples.
4. Administer the Synacthen as a push intravenously, followed by a sodium chloride 0.9% flush.
5. Administer the arginine via intravenous infusion over 30 minutes, straight after the administration of Synacthen. The time that the infusion STARTS (not finishes) is Time 0. Collection of the 30-minute samples for both the GHST and SST will need to be done immediately (following flush) after completion of the arginine infusion. Blood sampling at timepoints is as outlined in table below.

6. Administer the clonidine orally (dose as per dosing table above) as soon as the +90 Min blood sample has been collected.
7. Consider giving a bolus of 10 mL/kg IV of 0.9% sodium chloride over 30 minutes following the clonidine administration, to minimise the fall in blood pressure. **The clinician may choose to give a volume less than 10 mL/kg depending on how much volume was given at time of arginine infusion and size/age of the child.
8. Continue blood sampling at timepoints as outlined in table below.
9. Check a blood glucose level using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
10. For symptomatic hypotension during the test (> 30% fall in systolic BP from pre-test systolic BP or systolic BP < 80 mmHg) consider a further 10 mL / kg 0.9% sodium chloride bolus. If unsure or no response, call medical team for advice.
11. Take care ambulating the child following completion of the test. Postural hypotension may occur.
12. No food (other than for the treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge and have been observed for a minimum of 30 minutes following completion of the test. If observations are abnormal, repeat as required. A review by the medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 5 Administration, dose and sampling summary for Synacthen, arginine, and clonidine

Drug Administered			**Dose Administered**						**Time Administered**				
	Baseline		**Minutes post START of arginine infusion**										
Actual time bloods taken		Administer Synacthen and arginine						Administer clonidine					
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	240 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Cortisol	✓		✓		✓								
ACTH	✓												
Other tests, for example, IGF1, IGFBP3, as per requesting clinician	+/-												
Sample Tubes/ Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

Interpretation:

GHST Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies in different centres throughout the world and between paediatric and adult practices.

GH level cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the Pharmaceutical Benefits Scheme (PBS) in Australia or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by ther PBS and Pharmac.

Table 6 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube

Table 7 New Zealand: Biochemical Pharmac criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

Short Synacthen Test Interpretation

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in the inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off.

No definitive studies have been performed in the paediatric population to determine the cortisol response in healthy children using mass spectrometry-based methods.

The table below describes the minimum cortisol level achieved in healthy adults post IV Synacthen administration at 30 minutes for gas chromatography-mass spectrometry (GC-MS) and different immunoassays.

The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than those recorded at 30 minutes.

Table 9 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 and 60 minutes post IV Synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. GC-MS, gas chromatography-mass spectrometry; OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Cortisol level in neonates

In neonates <6 months, the initial sub-optimal cortisol responses (measured on the Roche GEN I assay on the Cobas e602 analyser) to Synacthen stimulation (defined as <550nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age and have higher 30-minute cortisol responses on initial testing (390 nmol/L vs 181 nmol/L).

SST Interpretation note

Although both 30-minute and 60-minute samples are recommended, the 30-minute cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Exercise caution in the interpretation of the cortisol response in patients on oestrogen therapy, such as the oral contraceptive pill (OCP), as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

REFERENCES

See individual protocols

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test (Glucagon and Arginine) and GnRH Stimulation Test (Triptorelin [Aus], Gonadorelin [NZ], or Buserelin [NZ])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post-neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for the tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Glucagon stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency
GnRH stimulation test	To assess the hypothalamic-pituitary-gonadal axis (LH, FSH and testosterone [males] or estradiol [females]) in suspected central precocious puberty or hypogonadotropic hypogonadism

Notes. ACTH, adrenocorticotropic hormone; FSH, follicle stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; LH, luteinising hormone

Arginine stimulates growth hormone (GH) secretion by (1) stimulation of α-adrenergic receptors and subsequent GH-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Glucagon stimulates GH secretion by stimulation of α-adrenergic receptors and subsequent GHRH release. It also causes a rise in blood glucose levels and subsequent insulin secretion which indirectly stimulates GH and adrenocorticotropic hormone (ACTH) secretion.

Contraindications:

Severe renal, cardiac, or liver disease

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce GH and cortisol response).

Failure to eat for 48 hours, or a glycogen storage disorder (GSD), or severe cortisol deficiency (in these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur).

Diabetes (the glucagon stimulation test is unreliable in individuals with diabetes as this GH ‘stimulus’ requires endogenous insulin).

Certain drugs, for example, periactin, that interfere with arginine stimulation. Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Known allergic tendencies

Pregnancy (a relative contraindication)

Precautions:

Ensure the patient has robust intravenous (IV) access for arginine infusion. Arginine can cause extravasation / chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient

Expertise level:

The minimum requirement is for the for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and the ability to site an IV cannula.

Formulation & Dose:

Table 2 Arginine formulation, dose and route of delivery

Formulation	**Dose**	**Route**
Arginine hydrochloride	0.5 grams / kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Glucagon formulation, dose and route of delivery

Formulation	Dose	Route
Glucagon hydrochloride (1 mg; powder plus diluent)	30 mcg/kg (max 1 mg)	Subcutaneous

Table 4 Triptorelin, gonadorelin, and buserelin – formulation, dose and route of delivery

Formulation	Dose	Route
Australia		
Triptorelin acetate (Decapeptyl 100 micrograms/mL) Note: DO NOT USE Diphereline (long-acting triptorelin)	100 micrograms/m2 or 2.5 micrograms/kg (max 100 micrograms)	Subcutaneous
New Zealand		
Gonadorelin (HRF, Ayerst, Factrel)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)
Buserelin acetate (Suprefact)* (1 mg/mL)	100 micrograms	Subcutaneous

Note. *Buserelin is used for the investigation of delayed puberty.

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis – extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after the administration of arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests – there have been case reports.

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of the arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Glucagon

Transient nausea, flushing, vomiting for 1–2 minutes, abdominal pain/cramps, or feelings of apprehension may occur.

Glucagon stimulates a 2–3-fold rise in blood glucose level following administration. This is maximal within the first hour. Following this rise in the blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Anaphylaxis is a very rare, but potential, complication.

GnRH agonist (triptorelin, gonadorelin, or buserelin)

Significant adverse reactions have not been encountered. Occasionally, subjects may experience nausea and abdominal pain.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Ensure the patient has robust IV access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

HOWEVER, as this combined test includes a gonadotropin-releasing hormone (GnRH) stimulation test to assess for precocious/delayed puberty, sex steroid priming should NOT be used for the GH stimulation component of this combined test as it will nullify the GnRH stimulation test.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, glucagon, triptorelin, gonadorelin, or buserelin

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test, unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps from the Preparation section have been taken prior to proceeding with the test.

2. Weigh the patient and take baseline observations.

3. Calculate and prescribe the arginine, glucagon, and triptorelin/gonadorelin doses.

4. Insert the IV cannula and take baseline (pre-stimulation) blood samples.

5. Administer the triptorelin or buserelin subcutaneously OR gonadorelin intravenously as a slow push over 1 minute.

6. Administer the glucagon subcutaneously (dose as per dosing table above) immediately following the administration of triptorelin, gonadorelin, or buserelin (dose/route as per dosing table below).

7. Administer the arginine via IV infusion over 30 minutes. The time that the infusion STARTS (not finishes) is Time 180 minutes. Ensure delivery of a 10–15 mL flush with sodium chloride 0.9% prior to taking the 210-minute blood sample.

8. Continue the blood sampling at the timepoints as outlined in the table below.

9. Check blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.

10. No food (other than for the treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge. If observations are abnormal, repeat as required. A review by the medical or nursing personnel (as per local practice) is to be undertaken prior to discharge.

Sample collection:

Table 5 Administration, dose and sampling summary – triptorelin, gonadorelin, buserelin, glucagon and arginine

Drug Administered				**Dose Administered**							**Time Administered**						
		Baseline		**Minutes post START of glucagon injection**													
Actual time bloods taken			Administer triptorelin/gonadorelin/ busrelin and glucagon								Administer arginine						
Test		-1 Min		30 min	45 min	60 Min	90 Min	120 Min	150 Min	180 Min		210 Min	225 Min	240 Min	255 Min	270 Min	24 Hr
GH		✓				✓	✓	✓	✓	✓			✓	✓	✓	✓	
Glucose		✓				✓	✓	✓	✓	✓			✓	✓	✓	✓	
LH and FSH	Triptorelin used	✓				✓		✓				✓		✓			
	Gonadorelin used	✓		✓	✓	✓	✓	✓									
	Buserelin used	✓												✓			
Testosterone (males) or oestradiol (females)	Triptorelin or Gonadorelin used	✓															✓
	Buserelin used	✓												✓			
Other tests, e.g., IGF1, IGFBP3, ACTH, or cortisol, at baseline and various timepoints as per requesting clinician		+/-															
Sample Tubes / Minimum Blood Volume		SST 2 mL				SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

See Notes section below ('Timing of post-triptorelin/gonadorelin stimulation blood sampling')

For the triptorelin and gonadorelin protocols, the 24-hour testosterone/oestradiol samples are usually included in the investigation of delayed puberty.

Buserelin is used for the investigation of delayed puberty in New Zealand

Interpretation:

GHST Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies across different centres throughout the world and between paediatric and adult practices.

GH cut-off thresholds that are currently in use range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the Pharmaceutical Benefits Scheme (PBS) in Australia or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of the end of 2022) GH cut-off thresholds used by the PBS and Pharmac.

Table 6 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube

Table 7 New Zealand: Biochemical PHARMAC criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD deficient sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

GnRH Stimulation Test Interpretation

An LH peak post-GnRH agonist of ≥5.0 IU/L with an LH-dominant response suggests hypothalamic-pituitary-gonadal (HPG) axis activation. This LH cut-off is the most widely accepted in the literature but is dependent on the assay used.

An LH peak post-GnRH agonist of <5.0 IU/L with an FSH-dominant response is supportive of premature thelarche, which may warrant continued monitoring of pubertal progression.

For buserelin, in the investigation of delayed puberty the following interpretations apply:

Based on the levels at 4 hours

LH and FSH peaks post-GnRH agonist of <5.0 IU/L – likely hypogonadotropic hypogonadism (LH-preferred)

LH and FSH peaks post-GnRH agonist of >10 IU/L – normal HPG axis

LH and FSH peak post-GnRH agonist of 5–10 IU/L – equivocal but probably normal HPG axis

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Timing of post-triptorelin/gonadorelin blood sampling note

The peak LH response has been reported to occur at various time points between 30 minutes to 180 minutes post-GnRH/GnRH agonist stimulation. This is dependent on the study design and the GnRH/GnRHa, sampling timepoints, and LH assay used.

If you are only taking blood samples at baseline and 1–2 timepoints post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, according to the available literature, the best time to take the stimulated LH sample(s) (i.e. the timepoint(s) with the best diagnostic accuracy for central precocious puberty) are:

Triptorelin studies: LH sample taken at either 30 minutes, 60 minutes, or 180 minutes post triptorelin administration

Gonadorelin studies: LH sample taken at either 30 minutes, 40 minutes, 45 minutes, or 60 minutes post gonadorelin administration

Please discuss with the consultant responsible for the patient at which timepoints they would like samples to be collected.

Some studies support the additional sampling timepoint of 24 hours post-GnRH/GnRHa stimulation for a testosterone/estradiol level, to improve the diagnostic accuracy of the test. Other studies report that this isn't required to rule in/rule out a diagnosis of central precocious puberty (CPP). Discuss with the consultant responsible for the patient about whether they would like this 24-hour blood sample taken.

Use of baseline LH levels for diagnostic purposes

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay-specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs before relying on baseline LH levels for the assessment of precocious puberty.

Use and interpretation of GnRH stimulation test in infants and pre-school aged children

Use of the GnRH stimulation test in young children to establish a diagnosis of CPP has its limitations with respect to the interpretation of results. A peak LH of >5.0 IU/L is commonly used as the diagnostic cut-off for CPP. However, in infants and pre-school-aged children, this peak LH cut-off level is likely too low.

In a Danish study of 48 healthy girls <6 years of age, assessed clinically to be pre-pubertal, the following LH and FSH responses, measured on the Roche Cobas e601 platform, were achieved at 30 minutes post gonadorelin IV injection (0.1 mg/m^2 body surface area, maximum dose 0.1 mg):

Table 8 post-gonadorelin LH and FSH responses

	Age group (years)					
	0–1	**1–2**	**2–3**	**3–4**	**4–5**	**5–6**
Stimulated LH (IU/L) Median (minimum, maximum)	7.57 (5.63–7.66)	4.86 (2.38–8.00)	4.31 (2.84–9.96)	2.19 (1.15–3.92)	3.74 (1.63–5.47)	2.61 (0.87–3.46)
Stimulated FSH (IU/L) Median (minimum, maximum)	26.56 (22.82–40.39)	20.51 (16.62–9.43)	20.14 (9.11–36.15)	12.15 (7.94–19.00)	17.22 (10.40–20.69)	11.53 (6.81–26.95)
Stimulated LH/FSH ratio Median (minimum, maximum)	0.21 (0.19–0.33)	0.25 (0.11–0.29)	0.21 (0.14–0.37)	0.16 (0.06–0.37)	0.26 (0.09–0.43)	0.19 (0.07–0.39)

Notes. FSH, follicle stimulating hormone; LH, luteinising hormone

During infancy, usually between 1–6 months of age, there is transient activation of the HPG axis, termed the ‘mini-puberty of infancy’. Performing a GnRH stimulation test duringthe mini-puberty of infancy will generate a positive result.

REFERENCES

See individual protocols.

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test (Arginine and Clonidine) and GnRH Stimulation Test (Triptorelin [Aus], Gonadorelin [NZ], or Buserelin [NZ])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post-neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Clonidine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
GnRH stimulation test	To assess the hypothalamic-pituitary-gonadal axis [LH, FSH and testosterone (males) or estradiol (females)] in suspected central precocious puberty or hypogonadotropic hypogonadism

Notes. ACTH, adrenocorticotropic hormone; GH, growth hormone

Arginine and clonidine stimulate growth hormone (GH) secretion by (1) stimulation of α-adrenergic receptors and subsequent GH-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Contraindications:

Severe renal, cardiac, or liver disease.

Sick sinus syndrome, compromised intravascular volume, hypotension, syncope, or autonomic dysfunction.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated adrenal insufficiency, hypothyroidism or panhypopituitarism (thyroxine deficiency may reduce GH response).

Certain drugs, for example, periactin, that interfere with arginine stimulation (note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated).

Known allergic tendencies.

Children with known congenital/acquired heart disease (exercise caution).

Pregnancy (a relative contraindication).

Precautions:

Ensure the patient has robust intravenous (IV) access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient

Expertise level:

The minimum requirement is for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and the ability to site an IV cannula.

Anaphylaxis to Synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation and Dose:

Table 2 Formulation, dose and route of delivery for arginine

Formulation	**Dose**	**Route**
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Formulation, dose and route of delivery for clonidine

Formulation	Dose	Route	Notes
Clonidine	100 micrograms/m2 (maximum 250 micrograms)	Oral	Calculate dose to nearest half tablet

Table 4 Formulation, dose and route of delivery – triptorelin, ganodorelin, and buserelin

Formulation	Dose	Route
Australia		
Triptorelin acetate solution (Decapeptyl 100 micrograms/mL) Note: DO NOT USE Diphereline depot injection (long-acting triptorelin)	100 micrograms/m2 (max 100 micrograms)	Subcutaneous
New Zealand		
Gonadorelin (HRF, Ayerst, Factrel)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)
Buserelin acetate (Suprefact)* (1 mg/mL)	100 micrograms	Subcutaneous

Note. *Buserelin is used for the investigation of delayed puberty.

Note:

Clonidine 100-microgram and 150-microgram tablets available on the Pharmaceutical Benefits Scheme (PBS) in Australia.

Clonidine 25-microgram and 150-microgram tablets are available in New Zealand.

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis – extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after the administration of arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests – there have been case reports..

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Clonidine

Drowsiness 1–3 hours post ingestion, nausea, and vomiting.

Hypotension, postural hypotension – fall in blood pressure by approximately 10 mmHg about 1 hour after ingestion which sually resolves by the end of the test but may last several hours. The effect is prolonged in renal failure. A bolus of 10 mL/kg 0.9% sodium chloride administered over 30 minutes following clonidine administration can minimise the fall in blood pressure.

GnRH (triptorelin, gonadorelin, or buserelin)

Significant adverse reactions have not been encountered. Occasionally, subjects may experience nausea and abdominal pain.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If patient is already on GH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Ensure the patient has robust IV access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

If on regular antihypertensive medication, please check with the consultant responsible for the patient about withholding this medication prior to the test.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Note: children with ADHD may be on clonidine treatment doses of up to 400 mcg daily. The clonidine should be withheld the day before and on the day of the test. Consideration should be given to using an alternative protocol, including either arginine or glucagon.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through Pharmaceutical Management Agency (Pharma) requires priming of all children at the age of 5–6 years for GH stimulation testing.

HOWEVER: as this combined test includes a gonadotropin-releasing hormone (GnRH) stimulation test to assess for precocious/delayed puberty, sex-steroid priming should NOT be used for the GH stimulation component of this combined test, as it will nullify the GnRH stimulation test.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, clonidine, triptorelin, gonadorelin, buserelin

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test, unless otherwise stipulated by the consultant or local policy.

Method:

1. Ensure the steps from the Preparation section have been undertaken prior to proceeding with the test. Ideally, perform the test first thing in the morning following an overnight fast (see fasting protocol for age-based maximum fasting durations). However, a minimum fasting time of only 2 hours is required, and this shorter fasting time should be applied in infants and young children.
2. Weigh the patient and take baseline observations.
3. Calculate and prescribe the arginine, clonidine, and triptorelin/gonadorelin/buserelin doses.
4. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer the triptorelin or buserelin subcutaneously OR the gonadorelin intravenously as a slow push over 1 minute.
6. Administer the arginine via IV infusion over 30 minutes immediately following the administration of triptorelin, gonadorelin, or buserelin (dose/route as per dosing table below). The time that the infusion STARTS (not finishes) is Time 0. Collection of the 30-minute samples for both the GHST and GnRH stimulation tests will need to be done immediately (following the flush) after completion of the arginine infusion.
7. Conduct the blood sampling at the timepoints as outlined in the table below
8. Administer the clonidine orally as soon as the +90-minute blood sample has been collected.
9. Continue blood sampling at the timepoints as outlined in the table below.
10. Check blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.

11. No food (other than for treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge; if abnormal, repeat as required. A review by the medical or nursing personnel (as per local practice) must be undertaken prior to discharge.

Sample collection:

Table 5 Administration, dose and sampling summary – arginine, triptorelin, gonadorelin, buserelin, and clonidine

Drug Administered				**Dose Administered**								**Time Administered**			
	Baseline		**Minutes post START of arginine infusion**												
Actual time bloods taken		Administer arginine, and triptorelin, gonadorelin, or buserelin						Administer clonidine							
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	240 Min	24 Hr
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	
Cortisol	✓		✓		✓									
ACTH	✓													
LH and FSH – Triptorelin used	✓		✓		✓				✓		✓			
LH and FSH – Gonadorelin used	✓		✓	✓	✓									
LH and FSH – Buserelin used	✓												✓	
Testosterone (males) or oestradiol (females) – Triptorelin or gonadorelin used	✓													✓
Testosterone (males) or oestradiol (females) – Buserelin used	✓												✓	
Other tests, e.g., IGF1, IGFBP3, ACTH, cortisol, as per requesting clinician	+/-													
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL							

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

For the triptorelin and gonadorelin protocols, the 24-hour testosterone/estradiol samples are usually included in the investigation of delayed puberty.

Buserelin is used for the investigation of delayed puberty in New Zealand.

GHST Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies in different centres throughout the world and between paediatric and adult practice.s

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the PBS in Australia or Pharma in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by PBS and Pharma.

Table 6 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GH deficiency, for example, septo-optic dysplasia, midline abnormality, genetically proven GH deficiency, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube

Table 7 New Zealand: Biochemical PHARMAC criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

GnRH Stimulation Test Interpretation

An LH peak post-GnRH agonist of ≥5.0 IU/L with an LH-dominant response suggests hypothalamic-pituitary-gonadal (HPG) axis activation. This LH cut-off is the most widely accepted in the literature but is dependent on the assay used.

An LH peak post-GnRH agonist of <5.0 IU/L with an FSH-dominant response is supportive of premature thelarche, which may warrant continued monitoring of pubertal progression.

For buserelin, in the investigation of delayed puberty the following interpretations apply:

Based on the levels at 4 hours

LH and FSH peaks post-GnRH agonist of <5.0 IU/L – likely hypogonadotropic hypogonadism (LH-preferred)

LH and FSH peaks post-GnRH agonist of >10 IU/L – normal HPG axis

LH and FSH peak post-GnRH agonist of 5–10 IU/L – equivocal but probably normal HPG axis

See Notes section below regarding the use and interpretation of GnRH stimulation test for the diagnosis of precocious puberty in children younger than 3 years old.

A complete lack of a gonadotropin response supports the diagnosis of hypogonadotropic hypogonadism, whereas a measurable but low response has limited predictive value (and may also occur in the constitutional delay of puberty).

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Effect of sex and/or Tanner stage on GnRH stimulation test results

Girls with signs of early puberty (Tanner stages 2 –3) who undergo a GnRH stimulation test as part of the assessment for central precocious puberty (CPP) may reach a reasonably low peak LH level during the GnRH stimulation test, while girls with CPP who have more advanced signs of puberty (Tanner stage > 3) and boys with CPP tend to have a brisker LH response. In the girls with early puberty, additional measures from the GnRH stimulation test that may assist with differentiating between CPP and idiopathic premature thelarche (IPT) are a peak LH/peak FSH ratio above a certain threshold and/or a 24-hour post-GnRH stimulation oestradiol level in the pubertal range.

Use of baseline LH levels for diagnostic purposes

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay-specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs before relying on baseline LH levels for the assessment of precocious puberty.

Timing of post-triptorelin/gonadorelin blood sampling

A peak LH response has been reported to occur at various time points between 30 minutes to 180 minutes post-GnRH/GnRH agonist stimulation. This is dependent on the study design AND the GnRH/GnRHa,sampling timepoints, and LH assay used.

If only taking blood samples at baseline and 1–2 timepoints post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, according to the available literature, the best time to take the stimulated LH sample(s) (i.e. the timepoint(s) with the best diagnostic accuracy for central precocious puberty) are:

Triptorelin studies: LH sample taken at either 30 minutes, 60 minutes, or 180 minutes post-triptorelin

Gonadorelin studies: LH sample taken at either 30 minutes, 40 minutes, 45 minutes or 60 minutes post-gonadorelin

Please discuss with the consultant responsible for the patient about which timepoints they would like samples to be taken.

Some studies support the additional sampling timepoint of 24 hours post-GnRH/GnRHa stimulation for a testosterone/oestradiol level to improve the diagnostic accuracy of the test for CPP. Other studies report that this is not required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRHa stimulation testosterone/oestradiol level can also be used in the assessment of delayed puberty. Discuss with the consultant responsible for the patient about whether they would like this 24-hour blood sample taken.

Use and interpretation of GnRH stimulation test in infants and pre-school aged children

Use of the GnRH stimulation test in young children to establish a diagnosis of CPP has its limitations with respect to the interpretation of results. A peak LH of >5.0 IU/L is commonly used as the diagnostic cut-off for CPP. However, in infants and pre-school aged children, this peak LH cut-off level is likely too low.

In a Danish study of 48 healthy girls <6 years of age, assessed clinically to be pre-pubertal, the following LH and FSH responses (see the table below), measured on the Roche Cobas e601 platform, were achieved at 30 minutes post gonadorelin intravenous injection (0.1 mg/m^2 body surface area, maximum dose 0.1 mg).

Table 8 Post-gonadorelin LH and FSH responses

	Age group (years)					
	0–1	**1–2**	**2–3**	**3–4**	**4–5**	**5–6**
Stimulated LH (IU/L) Median (minimum, maximum)	7.57 (5.63–7.66)	4.86 (2.38–8.00)	4.31 (2.84–9.96)	2.19 (1.15–3.92)	3.74 (1.63–5.47)	2.61 (0.87–3.46)
Stimulated FSH (IU/L) Median (minimum, maximum)	26.56 (22.82–40.39)	20.51 (16.62–29.43)	20.14 (9.11–36.15)	12.15 (7.94–19.00)	17.22 (10.40–20.69)	11.53 (6.81–26.95)
Stimulated LH/FSH ratio Median (minimum, maximum)	0.21 (0.19–0.33)	0.25 (0.11–0.29)	0.21 (0.14–0.37)	0.16 (0.06–0.37)	0.26 (0.09–0.43)	0.19 (0.07–0.39)

Notes. FSH, follicle stimulating hormone; LH, luteinising hormone

During infancy, usually between 1–6 months of age, there is transient activation of the HPG axis, termed the ‘mini-puberty of infancy’. Performing a GnRH stimulation test during the mini-puberty of infancy will generate a positive result.

REFERENCES

See individual protocols.

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test (Glucagon and Arginine), GnRH Stimulation Test (Triptorelin [Aus], Gonadorelin [NZ], or Buserelin [NZ]), and Short Synacthen Test (Synacthen [ACTH])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post-neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Glucagon stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency
GnRH stimulation test	To assess the hypothalamic-pituitary-gonadal axis (LH, FSH and testosterone [males] or estradiol [females]) in suspected central precocious puberty or hypogonadotropic hypogonadism
Short synacthen test	To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency

Notes. ACTH, adrenocorticotropic hormone; FSH, follicle stimulating hormone; GH, growth hormone; LH, luteinising hormone

Arginine stimulates GH secretion by (1) stimulation of α-adrenergic receptors and subsequent GH-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Glucagon stimulates GH secretion by stimulation of α-adrenergic receptors and subsequent GHRH release. It also causes a rise in blood glucose levels and subsequent insulin secretion which indirectly stimulates GH and adrenocorticotropic hormone (ACTH) secretion.

Contraindications:

Severe renal, cardiac, or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride)

Recent or current acute illness.

Untreated hypothyroidism or hypocortisolism (thyroxine deficiency may reduce the GH and cortisol response).

Failure to eat for 48 hours, or a glycogen storage disorder (GSD), or severe cortisol deficiency (in these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur).

Diabetes (the glucagon stimulation test is unreliable in individuals with diabetes as this GH 'stimulus' requires endogenous insulin).

Certain drugs, for example, periactin, interfere with arginine stimulation. Note, it is recommended that potential interactions with drug concomitantly taken by the patient be investigated.

Known allergic tendencies.

Pregnancy (a relative contraindication)

Known hypersensitivity to ACTH.

Ongoing treatment with Synacthen only.

Current treatment with supraphysiological doses of glucocorticoids.

Precautions:

Ensure the patient has robust intravenous (IV) access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after the arginine infusion will be invalid

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and the ability to site an IV cannula.

Anaphylaxis to Synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation & Dose:

Table 2 Arginine formulation, dose and route of delivery

Formulation	Dose	Route
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Glucagon formulation, dose and route of delivery

Formulation	Dose	Route
Glucagon hydrochloride (1 mg; powder and diluent)	30 mcg/kg (max 1 mg)	Subcutaneous

Table 4 Formulation, dose and route of delivery – triptorelin, gonadorelin, buserelin

Formulation	Dose	Route
Australia		
Triptorelin acetate (Decapeptyl 100 micrograms/mL) Note: DO NOT USE Diphereline (long-acting triptorelin)	100 micrograms/m2 or 2.5 micrograms/kg (max 100 micrograms)	Subcutaneous
New Zealand		
Gonadorelin (HRF, Ayerst, Factrel)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)
Buserelin acetate (Suprefact)* (1 mg/mL)	100 micrograms	Subcutaneous

Note. *Buserelin is used for the investigation of delayed puberty

Table 5 Synacthen formulation, dose and route of delivery

Formulation	Dose		Route
Tetracosactide (Synacthen, solution for injection) 250 mcg in 1 mL	0–6 months old	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis – extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after the administration of arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests. – there have been case reports.

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Glucagon

Transient nausea, flushing, vomiting for 1–2 minutes, abdominal pain/cramps, and feelings of apprehension may occur.

Glucagon stimulates a 2–3 fold-rise in the blood glucose level following administration. This is maximal within the first hour. Following this rise in the blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Anaphylaxis is a very rare, but potential, complication

GnRH (triptorelin, gonadorelin, or buserelin)

Significant adverse reactions have not been encountered. Occasionally subjects may experience nausea and abdominal pain.

Synacthen

Hypersensitivity or anaphylactic reactions are rare. Patients may experience dizziness and nausea.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function test (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If the patient is already onGH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Ensure the patient has robust IV access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed before undertaking a short Synacthen test (SST). For individuals on physiological or sub-physiological glucocorticoid doses or short courses of supraphysiological doses of glucocorticoids, withhold the glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

The test can be performed any time of the day but preferably before 09:00 in order to appropriately assess the basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the SST), then the SST can be performed at any time of day, as the peak cortisol level following ACTH (Synacthen) stimulation will still be measurable.

In patients who have recently undergone neurosurgery and are at risk of ACTH deficiency (secondary adrenal insufficiency), check with the consultant responsible for the patient about the desired timeframe post-surgery for arranging the SST. Following the loss of endogenous ACTH supply, the adrenal glands will eventually atrophy and no longer be able to produce adequate cortisol levels. However, this process takes time, and in the first approximately 6 weeks after the onset of ACTH deficiency (as a result of neurosurgery), the adrenal glands will still be able to produce an adequate (normal), but falsely reassuring, response to exogenous ACTH (synacthen) during an SST. A low early morning (basal) cortisol level during this time can suggest that ACTH deficiency (secondary adrenal insufficiency) is likely. Until the ACTH status of patients at risk of ACTH deficiency is known, they should have a plan in place for stress steroid cover during times of illness, further surgery, or other stressors.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through the Pharmaceutical Management Agency (Pharma) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

HOWEVER: as this combined test includes a gonadotropin-releasing hormone (GnRH) stimulation test to assess for precocious/delayed puberty, sex-steroid priming should NOT be used for the GH stimulation component of this combined test, as it will nullify the GnRH stimulation test.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, glucagon, triptorelin, gonadorelin or buserelin, synacthen

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test**,** unless stipulated otherwise by the consultant or local policy.

Method:

1. Ensure the steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh the patient and take baseline observations.
3. Calculate and prescribe arginine, glucagon, triptorelin/gonadorelin/buserelin, and Synacthen doses.
4. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer the triptorelin or buserelin subcutaneously OR the gonadorelin intravenously as a slow push over 1 minute.
6. Administer the glucagon subcutaneously (dose as per the dosing table above) immediately following administration of the triptorelin, gonadorelin, or buserelin (dose/route as per the dosing table below).
7. Administer the Synacthen as a slow IV push.
8. Immediately after the administration of Synacthen, administer arginine via IV infusion over 30 minutes. The time that the infusion STARTS (not finishes) is Time 180 minutes. Ensure a 10–15-mL flush with sodium chloride 0.9% prior to taking the 210-minute blood sample.
9. Continue blood sampling at timepoints, as outlined in the able below.
10. Check blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit’s hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
11. No food (other than for treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge. If abnormal, repeat as required. A review by the medical or nursing personnel (as per local practice) must be undertaken prior to discharge.

Sample collection:

Table 6 Administration, dose and sampling summary – triptorelin/gonadorelin/buserelin and Synacthen/arginine

Drug Administered					**Dose Administered**						**Time Administered**					
		Baseline			**Minutes post START of glucagon injection**											
Actual time bloods taken			Administer triptorelin/gonadorelin/ buserelin and glucagon							Administer Synacthen and arginine						
Test		-1 Min		30 min	45 min	60 Min	90 Min	120 Min	150 Min	180 Min		210 Min	225 Min	240 Min	255 Min	270 Min	24 Hr
GH		✓				✓	✓	✓	✓	✓			✓	✓	✓	✓	
Glucose		✓				✓	✓	✓	✓	✓			✓	✓	✓	✓	
Cortisol		✓										✓		✓			
ACTH		✓															
LH and FSH	Triptorelin used	✓				✓		✓				✓		✓			
	Gonadorelin used	✓		✓	✓	✓	✓	✓									
	Buserelin used	✓												✓			
Testosterone (males) or Estradiol (females)	Triptorelin or gonadorelin used	✓															✓
	Buserelin used	✓												✓			
Other tests, e.g., IGF1, IGFBP3, ACTH, cortisol at baseline and various timepoints, as per requesting clinician		+/-															
Sample Tubes / Minimum Blood Volume		SST 2 mL				SST1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL		SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL	SST 1 mL

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

For the triptorelin and gonadorelin protocols, the 24-hour testosterone/oestradiol samples are usually included in the investigation of delayed puberty.

Buserelin is used for the investigation of delayed puberty in New Zealand.

GHST Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies in different centres throughout the world and between paediatric and adult practice.s

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the Pharmaceutical Benefits Scheme (PBS) in Australia or the Pharma in New Zealand.

Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharma.

Table 7 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic insulin tolerance test with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH <3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube

Table 8 New Zealand: Biochemical PHARMAC criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5 mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

GnRH Stimulation Test Interpretation

An LH peak post-GnRH agonist of ≥5.0 IU/L with an LH-dominant response suggests hypothalamic-pituitary-gonadal (HPG) axis activation. This LH cut-off is the most widely accepted in the literature but is dependent on the assay used.

An LH peak post-GnRH agonist of <5.0 IU/L with an FSH-dominant response is supportive of premature thelarche, which may warrant continued monitoring of pubertal progression.

For buserelin, in the investigation of delayed puberty the following interpretations apply:

Based on the levels at 4 hours

LH and FSH peaks post-GnRH agonist of <5.0 IU/L – likely hypogonadotropic hypogonadism (LH-preferred)

LH and FSH peaks post-GnRH agonist of >10 IU/L – normal HPG axis

LH and FSH peak post-GnRH agonist of 5–10 IU/L – equivocal but probably normal HPG axis

See Notes section below regarding the use and interpretation of GnRH stimulation test for the diagnosis of precocious puberty in children younger than 3 years old.

A complete lack of a gonadotropin response supports the diagnosis of hypogonadotropic hypogonadism, whereas a measurable but low response has limited predictive value (and may also occur in the constitutional delay of puberty).

Short Synacthen Test Interpretation

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in an inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off.

No definitive studies have been performed in the paediatric population to determine the cortisol response in healthy children using mass spectrometry-based methods.

The table below describes the minimum cortisol level achieved in healthy adults post IV Synacthen administration at 30 minutes for gas chromatography-mass spectrometry (GC-MS) and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than those recorded at 30 minutes.

Table 9 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 and 60 minutes post IV Synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. GC-MS, gas chromatography-mass spectrometry; OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency.

Cortisol level in neonates

In neonates <6 months, the initial sub-optimal cortisol responses (measured on the Roche GEN I assay on the Cobas e602 analyser) to Synacthen stimulation (defined as <550nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age and have higher 30-minute cortisol responses on initial testing (390 nmol/L vs 181 nmol/L).

SST interpretation note

Exercise caution in the interpretation of the cortisol response in patients on oestrogen therapy, such as the oral contraceptive pill (OCP), as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Effect of sex and/or Tanner stage on GnRH stimulation test results

Girls with signs of early puberty (Tanner stages 2 –3) who undergo a GnRH stimulation test as part of the assessment for CPP may reach a reasonably low peak LH level during the GnRH stimulation test, while girls with CPP who have more advanced signs of puberty (Tanner stage >3) and boys with CPP tend to have a brisker LH response. In the girls with early puberty, additional measures from the GnRH stimulation test that may assist with differentiating between CPP and idiopathic premature thelarche (IPT) are a peak LH/peak FSH ratio above a certain threshold and/or a 24-hour post-GnRH stimulation oestradiol level in the pubertal range.

Use of baseline LH levels for diagnostic purposes

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs before relying on baseline LH levels for the assessment of precocious puberty.

Timing of post-triptorelin/gonadorelin blood sampling

A peak LH response has been reported to occur at various time points between 30 minutes to 180 minutes post-GnRH/GnRH agonist stimulation. This is dependent on the study design and the the GnRH/GnRHa, sampling timepoints, and LH assay used.

If only taking blood samples at baseline and 1–2 timepoints post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, according to the available literature, the best time to take the stimulated LH sample(s) (i.e. the timepoint(s) with the best diagnostic accuracy for central precocious puberty) are:

> *Triptorelin studies*: LH sample taken at either 30 minutes, 60 minutes, or 180 minutes post-triptorelin
>
> *Gonadorelin studies*: LH sample taken at either 30 minutes, 40 minutes, 45 minutes or 60 minutes post-gonadorelin

Please discuss with the consultant responsible for the patient about which timepoints they would like samples to be taken.

Some studies support the additional sampling timepoint of 24 hours post-GnRH/GnRHa stimulation for a testosterone/oestradiol level to improve the diagnostic accuracy of the test. Other studies report that this is not required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRHa stimulation testosterone/oestradiol level can also be used in the assessment of delayed puberty. Discuss with the consultant responsible for the patient about whether they would like this 24-hour blood sample taken.

Use and interpretation of GnRH stimulation test in infants and pre-school aged children

Use of the GnRH stimulation test in young children to establish a diagnosis of CPP has its limitations with respect to the interpretation of results. A peak LH of >5.0 IU/L is commonly used as the diagnostic cut-off for CPP. However, in infants and pre-school aged children, this peak LH cut-off level is likely too low.

In a Danish study of 48 healthy girls <6 years of age, assessed clinically to be pre-pubertal, the following LH and FSH responses (see the table below), measured on the Roche Cobas e601 platform, were achieved at 30 minutes post gonadorelin IV injection (0.1 mg/m^2 body surface area, maximum dose 0.1 mg).

Table 10 Post-gonadorelin LH and FSH responses

	Age group (years)					
	0–1	**1–2**	**2–3**	**3–4**	**4–5**	**5–6**
Stimulated LH (IU/L) Median (minimum, maximum)	7.57 (5.63–7.66)	4.86 (2.38–8.00)	4.31 (2.84–9.96)	2.19 (1.15–3.92)	3.74 (1.63–5.47)	2.61 (0.87–3.46)
Stimulated FSH (IU/L) Median (minimum, maximum)	26.56 (22.82–40.39)	20.51 (16.62–29.43)	20.14 (9.11–36.15)	12.15 (7.94–19.00)	17.22 (10.40–20.69)	11.53 (6.81–26.95)
Stimulated LH/FSH ratio Median (minimum, maximum)	0.21 (0.19–0.33)	0.25 (0.11–0.29)	0.21 (0.14–0.37)	0.16 (0.06–0.37)	0.26 (0.09–0.43)	0.19 (0.07–0.39)

Notes. FSH, follicle stimulating hormone; LH, luteinising hormone

During infancy, usually between 1–6 months of age, there is transient activation of the HPG axis, termed the ‘mini-puberty of infancy’. Performing a GnRH stimulation test during the mini-puberty of infancy will generate a positive result.

REFERENCES

See individual protocols.

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test (Arginine and Clonidine), GnRH Stimulation Test (Triptorelin [Aus], Gonadorelin [NZ] or Buserelin [NZ]), and Short Synacthen Test (Synacthen[ACTH])

Indications:

When there are multiple pituitary hormone deficiencies suspected; this could include deficiencies due to a central nervous system (CNS) tumour, due to post-neurosurgery, following other insults to the hypothalamic-pituitary region, or when previous investigations suggest that one or more pituitary hormone deficiencies may be present.

Rationale:

There are several hypothalamus-pituitary-end organ axes. The table below outlines the rationale for each of the tests performed within this combined protocol.

Table 1 Rationale for tests in combined protocol

Test	Rationale
Arginine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
Clonidine stimulation test	To assess the anterior pituitary's ability to produce GH in suspected GH deficiency
GnRH stimulation test	To assess the hypothalamic-pituitary-gonadal axis (LH, FSH and testosterone [males] or estradiol [females]) in suspected central precocious puberty or hypogonadotropic hypogonadism
Short Synacthen test	To assess the hypothalamic-pituitary-adrenal axis (ACTH, cortisol) in suspected secondary adrenal insufficiency

Notes. ACTH, adrenocorticotropic hormone; GH, growth hormone

Arginine and clonidine stimulate growth hormone (GH) secretion by (1) stimulation of α-adrenergic receptors and subsequent GH-releasing hormone (GHRH) release, and (2) suppression of somatostatin.

Contraindications:

Severe renal, cardiac. or liver disease.

Electrolyte imbalance, especially hyperchloraemia or acidosis (arginine contains a significant amount of nitrogen and chloride).

Recent or current acute illness.

Untreated adrenal insufficiency, hypothyroidism, or panhypopituitarism (thyroxine deficiency may reduce the GH and cortisol response).

Certain drugs, for example, periactin, that interfere with arginine stimulation. Note, it is recommended that potential interactions with drugs concomitantly taken by the patient be investigated.

Known allergic tendencies.

Sick sinus syndrome, compromised intravascular volume, hypotension, syncope, or autonomic dysfunction.

Known congenital/acquired heart disease in children – exercise caution.

Known hypersensitivity to ACTH.

Ongoing treatment with Synacthen only.

Current treatment with supraphysiological doses of glucocorticoids.

Pregnancy (a relative contraindication).

Precautions:

Ensure the patient has robust intravenous (IV) access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

Prolongation of the arginine infusion period may result in diminished stimulation to the pituitary gland and nullification of the GH stimulation test (GHST).

Any urine testing for amino acids <24 hours after the arginine infusion will be invalid.

In infants and children younger than 4 years old, moderate hypoglycaemia may follow either glucagon or arginine stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and the ability to site an IV cannula.

Anaphylaxis to Synacthen has been reported but is rare. This test should be performed in clinical areas with full resuscitation facilities and staff trained in paediatric resuscitation.

Formulation and dose:

Table 2 Formulation, dose and route of delivery for arginine

Formulation	Dose	Route
Arginine hydrochloride	0.5 grams/kg (max 30 grams) Use a 10% solution: This may be available as a pre-made solution OR dilute arginine in 0.9% sodium chloride to make a 10% solution (10 grams arginine per 100 mL 0.9% sodium chloride) The dose in mL = 5 mL/kg (max 300 mL)	Intravenous infusion over 30 minutes

Table 3 Formulation, dose and route of delivery for clonidine

Formulation	Dose	Route	Notes
Clonidine	100 micrograms / m2 orally (maximum 250 micrograms)	Oral	Calculate dose to nearest half tablet

Table 4 Formulation, dose and route of delivery – triptorelin, gonadorelin, or buserelin

Formulation	Dose	Route
Australia		
Triptorelin acetate (Decapeptyl 100 micrograms/mL) Note: DO NOT USE Diphereline (long-acting triptorelin)	100 micrograms/m2 or 2.5 micrograms/kg (max 100 micrograms)	Subcutaneous
New Zealand		
Gonadorelin (HRF, Ayerst, Factrel)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)
Buserelin acetate (Suprefact)* (1 mg/mL)	100 micrograms	Subcutaneous

Note. *Buserelin is used for the investigation of delayed puberty.

Table 5 Formulation, dose, and route of delivery for Synacthen

Formulation	Dose		Route
Tetracosactide (Synacthen, solution for injection) 250 mcg in 1 mL	0–6 months old	15 mcg/kg (minimum 75 mcg to maximum of 125 mcg)	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Note:

Clonidine 100-microgram and 150-microgram tablets are available on the Pharmaceutical Benefits Scheme (PBS) in Australia.

Clonidine 25-microgram and 150-microgram tablets are available in New Zealand.

Adverse reactions:

Arginine

Rapid IV infusion may cause flushing, nausea, vomiting, numbness, headache, hypotension, and local venous irritation.

Allergic reactions and/or anaphylaxis are extremely rare; hypotension requiring IV fluid replacement has been rarely observed one hour after administration of the arginine infusion.

Elevated potassium in uraemic patients.

Transient haematuria following arginine stimulation tests – there have been case reports.

Hypoglycaemia in children – this can be a result of fasting prior to the test. It is also important to ensure that the correct dose of arginine is given (not an excessive dose), particularly if hypopituitarism is suspected in small infants, as excess arginine may provoke severe hypoglycaemia.

Note: care should be given to ensuring the correct dilution of the arginine, as deaths have been reported with dilution errors and patients who have received 10 times the intended dose.

Clonidine

Drowsiness 1–3 hours post ingestion, nausea, and vomiting.

Hypotension, postural hypotension – a fall in blood pressure by approximately 10 mmHg about 1 hour after ingestion which usually resolves by the end of the test but may last several hours. The effect is prolonged in renal failure. The administration of a bolus of 10 mL/kg 0.9% sodium chloride bolus over 30 minutes following clonidine administration can minimise the fall in blood pressure.

GnRH (Triptorelin, Gonadorelin, or Buserelin)

Significant adverse reactions have not been encountered. Occasionally subjects may experience nausea and abdominal pain.

Synacthen

Hypersensitivity or anaphylactic reactions are rare. Patients may experience dizziness and nausea.

Preparation:

Ensure the patient is euthyroid and has normal thyroid function tests (TFTs) prior to commencing the test.

Ensure the patient has normal electrolytes prior to commencing the test.

Overnight fast (see fasting protocol for age-based maximum fasting durations). Water is permitted.

If the patient is already onGH, this should ideally be ceased at least 96 hours (for daily recombinant growth hormone [rhGH]) or four weeks (for weekly rhGH) prior to the GHST.

Ensure the patient has robust IV access for the arginine infusion. Arginine can cause extravasation/chemical burn injury if not administered correctly.

If on regular antihypertensive medication, please check with the consultant responsible for the patient about withholding this medication prior to the test.

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed before undertaking a short Synacthen test (SST). For individuals on physiological or sub-physiological glucocorticoid doses or short courses of supraphysiological doses of glucocorticoids, withhold the glucocorticoids for 24 hours (48–72 hours in the case of dexamethasone) prior to testing (the child must be well) under medical supervision to avoid false positives. Check with the laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

The test can be performed any time of the day but preferably before 09:00 in order to appropriately assess the basal (early morning) cortisol secretion. However, if the patient has had an early morning basal cortisol sample performed recently (prior to the SST), then the SST can be performed at any time of day, as the peak cortisol level following ACTH (Synacthen) stimulation will still be measurable.

In patients who have recently undergone neurosurgery and are at risk of ACTH deficiency (secondary adrenal insufficiency), check with the consultant responsible for the patient about the desired timeframe post-surgery for arranging the SST. Following the loss of endogenous ACTH supply, the adrenal glands will eventually atrophy and no longer be able to produce adequate cortisol levels. However, this process takes time, and in the first approximately 6 weeks after the onset of ACTH deficiency (as a result of neurosurgery), the adrenal glands will still be able to produce an adequate (normal), but falsely reassuring, response to exogenous ACTH (Synacthen) during an SST. A low early-morning (basal) cortisol level during this time can suggest that ACTH deficiency (secondary adrenal insufficiency) is likely. Until the ACTH status of patients at risk of ACTH deficiency is known, there should be a plan in place for stress steroid cover during times of illness, further surgery, or other stressors.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on the request form.

Note: children with ADHD may be on clonidine treatment doses of up to 400 mcg daily. The clonidine should be withheld the day before and on the day of the test. Consideration should be given to using an alternative protocol, including either arginine or glucagon.

Sex-steroid priming

The question of sex-steroid priming for GH testing remains controversial, with no clear evidence or consensus yet emerging. The clearest rationale for priming is in boys or girls with delayed puberty – girls 12 years and above or boys 13 years and above without significant puberty. Strong consideration should be given to sex-steroid priming in these groups. Some paediatric endocrinologists consider sex-steroid priming in all boys and girls of 8 years and above, and this can also be considered a reasonable approach. Where sex-steroid priming is used, evidence favours the use of oestrogen in girls and boys, with no convincing rationale for the use of testosterone.

In New Zealand, the eligibility criteria for GH through the Pharmaceutical Management Agency (Pharmac) requires the priming of all children at the age of 5–6 years for GH stimulation testing.

HOWEVER: as this combined test includes a gonadotropin-releasing hormone (GnRH) stimulation test to assess for central precocious puberty, sex-steroid priming should NOT be used for the GH stimulation component of this combined test as it will nullify the GnRH stimulation test.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulants – arginine, clonidine, triptorelin, gonadorelin, buserelin, synacthen

Observations:

Observe the temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR) at baseline and then every 15 minutes throughout the test, unless stipulated otherwise by the consultant or local policy.

Method:

- Ensure the steps from the Preparation section have been undertaken prior to proceeding with the test. Ideally, perform the test first thing in the morning following an overnight fast (see fasting protocol for age-based maximum fasting durations). However, a minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.

2. Weigh the patient and take baseline observations.
3. Calculate and prescribe the arginine, clonidine, triptorelin/gonadorelin/buserelin, and Synacthen doses.
4. Insert the IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer the Synacthen, triptorelin/gonadorelin/buserelin and arginine, one after the other as follows –

   First: Synacthen intravenously as a push

   Second: triptorelin or buserelin subcutaneously OR gonadorelin intravenously as a slow push over 1 minute

   Third: arginine via intravenous infusion over 30 minutes

   The time that the arginine infusion STARTS (not finishes) is Time 0. Allow time to administer a 10–15-mL flush with sodium chloride 0.9% prior to taking the 30-minute blood sample.
6. Conduct blood sampling at the timepoints as outlined in the table below.
7. Administer the clonidine orally as soon as the +90-minute blood sample has been collected.
8. Continue blood sampling at the timepoints as outlined in the table below.
9. Check blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. After hypoglycaemia correction, you can continue with collecting the remaining samples for the stimulation test.
10. No food (other than for treatment of hypoglycaemia) is permitted until the test is completed. Water is permitted.

Discharge:

The child must have been fed and have normal observations and blood glucose level at the time of discharge; if abnormal, repeat as required. A review by the medical or nursing personnel (as per local practice) must be undertaken prior to discharge.

Sample collection:

Table 6 Administration, dose and route of delivery for Synacthen, triptorelin/gonadorelin/buserelin, arginine, and clonidine

Drug Administered				**Dose Administered**								**Time Administered**				
		Baseline		**Minutes post START of arginine infusion**												
Actual time bloods taken			Administer Synacthen, triptorelin/gonadorelin/ buserelin, and arginine						Administer clonidine							
Test		-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	240 Min	24 Hr
GH		✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	
Glucose		✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	
Cortisol		✓		✓		✓									
ACTH		✓													
LH and FSH	Triptorelin used	✓		✓		✓				✓		✓			
	Gonadorelin used	✓		✓	✓	✓									
	Buserelin used	✓												✓	
Testosterone (males) or Estradiol (females)	Triptorelin or gonadorelin	✓													✓
	Buserelin	✓												✓	
Other tests, e.g., IGF1, IGFBP3, ACTH, cortisol, as per requesting clinician		+/-													
Sample Tubes / Minimum Blood Volume															

Note. ACTH, adrenocorticotropic hormone; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; SST, serum separator tube.

For the triptorelin and gonadorelin protocols, the 24-hour testosterone/estradiol samples are usually included in the investigation of delayed puberty.

Buserelin is used for the investigation of delayed puberty in New Zealand.

GHST Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency (GHD) varies in different centres throughout the world and between paediatric and adult practices.

The GH cut-off thresholds that are currently in use for diagnosing GHD range from GH <0.4 mcg/L to GH <10 mcg/L.

To access funded GH treatment in Australia and New Zealand, there are different criteria that must be met, and these are determined by the PBS in Australia or Pharmac in New Zealand. Please check the relevant website(s) for these criteria, as they are updated and changed intermittently. Below is a summary of the current (as of 2023) GH cut-off thresholds used by the PBS and Pharmac.

Table 7 Australia: Biochemical PBS criteria for biochemical GHD

Children	Adults
Peak serum GH <3.3 mcg/L (<10 mU/L) in response to: • 2 pharmacological GHSTs, for example, arginine, clonidine, glucagon, insulin, OR • 1 pharmacological and 1 physiological GHST, for example, sleep, exercise, OR • 1 GHST (pharmacological or physiological) with other evidence of GHD, for example, septo-optic dysplasia, midline abnormality, genetically proven GHD, OR • 1 GHST (pharmacological or physiological) and low plasma IGF-1 levels OR • 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	Current or historical evidence of a diagnostic ITT with maximum serum GH <2.5 mcg/L OR Current or historical evidence of a diagnostic arginine infusion test with maximum serum GH <0.4 mcg/L OR Current or historical evidence of a diagnostic glucagon provocation test with maximum serum GH< 3 mcg/L

Note. GH, growth hormone; GHD, growth hormone deficiency; GHST, growth hormone stimulation test; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; ITT, insulin tolerance test; SST, serum separator tube

Table 8 New Zealand: Biochemical PHARMAC criteria for biochemical GHD

Children	Adults
GHD causing symptomatic hypoglycaemia, or with other significant GHD sequelae (for example, cardiomyopathy, hepatic dysfunction) and diagnosed with GH <5mcg/L on at least two random blood samples in the first 2 weeks of life, or from sampling during established hypoglycaemia (whole blood glucose <2 mmol/L using a laboratory device) OR Peak serum GH <5.0 mcg/L in response to 2 different GHSTs. In children who are 5 years and older, GH testing with sex-steroid priming is required.	For adults and adolescents, severe GHD is defined as peak serum GH level ≤3 mcg/L during an adequately performed ITT or glucagon stimulation test. Patients with one or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHSTs, of which one should be ITT unless contraindicated. Where an additional test is required, an arginine provocation test can be used with a peak serum GH ≤0.4 mcg/L.

Note. GHD, growth hormone deficiency; GHST, growth hormone stimulation test; ITT, insulin tolerance test

GnRH Stimulation Test Interpretation

An LH peak post-GnRH agonist of ≥5.0 IU/L with an LH-dominant response suggests hypothalamic-pituitary-gonadal (HPG) axis activation. This LH cut-off is the most widely accepted in the literature but is dependent on the assay used.

An LH peak post-GnRH agonist of <5.0 IU/L with an FSH-dominant response is supportive of premature thelarche, which may warrant continued monitoring of pubertal progression.

For buserelin, in the investigation of delayed puberty the following interpretations apply:

Based on the levels at 4 hours

LH and FSH peaks post-GnRH agonist of <5.0 IU/L – likely hypogonadotropic hypogonadism (LH-preferred)

LH and FSH peaks post-GnRH agonist of >10 IU/L – normal HPG axis

LH and FSH peak post-GnRH agonist of 5–10 IU/L – equivocal but probably normal HPG axis

See Notes section below regarding the use and interpretation of GnRH stimulation test for the diagnosis of precocious puberty in children younger than 3 years old.

A complete lack of a gonadotropin response supports the diagnosis of hypogonadotropic hypogonadism, whereas a measurable but low response has limited predictive value (and may also occur in the constitutional delay of puberty).

Short Synacthen Test Interpretation

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in an inappropriate over-diagnosis of adrenal insufficiency. Laboratories need to determine their own individual cut-off.

No definitive studies have been performed in the paediatric population to determine cortisol response in healthy children using mass spectrometry-based methods.

The table below describes the minimum cortisol level achieved in healthy adults post IV Synacthen at 30 minutes for gas chromatography-mass spectrometry (GC-MS) and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than the 30-minute levels.

Table 9 Minimal cortisol levels achieved in healthy adults post IV Synacthen for GC-MS and immunoassay

	Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 and 60 minutes post IV Synacthen. 60-minute values are based on the average rise of 15% from the 30-minute cortisol concentrations					
Cortisol Assay (nmol/L)	**Male**		**Female**		**Female (OCP)**	
	30 min	60 min	30 min	60 min	30 min	60 min
GC-MS	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Roche E170	420	483	420	483	640	736
Abbott Architect	430	495	420	483	580	667
Siemen Centaur	450	518	450	518	620	713
Siemen Immulite	470	541	480	552	690	794

Notes. GC-MS, gas chromatography-mass spectrometry; OCP, oral contraceptive pill; This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA). Although both 30-min and 60-min samples are recommended, the 30-min cortisol level is less reliable and cannot be used in isolation to make a diagnosis of adrenal insufficiency

Cortisol level in neonates

In neonates <6 months,the initial sub-optimal cortisol responses (measured on the Roche GEN I assay on the Cobas e602 analyser) to Synacthen stimulation (defined as <550nmol/L at 30 minutes) are often found to be transient on repeat testing. Those with a transient abnormality are likely to be small for gestational age and have higher 30-minute cortisol responses on initial testing (390 nmol/L vs 181 nmol/L).

SST interpretation

Exercise caution in the interpretation of the cortisol response in patients on oestrogen therapy such as the oral contraceptive pill (OCP), as this may result in higher cortisol levels associated with increased corticosteroid-binding globulin (CBG) levels.

We do NOT subscribe to historical requirements for a cortisol rise above a certain threshold in addition to a sufficient cortisol response. Some normal individuals with high baseline cortisol level may not achieve this rise.

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Effect of sex and/or Tanner stage on GnRH stimulation test results

Girls with signs of early puberty (Tanner stages 2–3) who undergo a GnRH stimulation test as part of the assessment for central precocious puberty (CPP) may reach a reasonably low peak LH level during the GnRH stimulation test, while girls with CPP who have more advanced signs of puberty (Tanner stage >3) and boys with CPP tend to have a brisker LH response. In the girls with early puberty, additional measures from the GnRH stimulation test that may assist with differentiating between CPP and idiopathic premature thelarche (IPT) are a peak LH/peak FSH ratio above a certain threshold and/or a 24-hour post-GnRH stimulation oestradiol level in the pubertal range.

Use of baseline LH levels for diagnostic purposes

There have been numerous studies investigating the value of baseline (non-stimulated) gonadotrophins in predicting responses following GnRH stimulation. Most are assay-specific with a wide range of sensitivity and specificity at various cut-offs. Generally, a baseline LH level of >0.2–0.3 IU/L has been reported to be predictive of a pubertal response. However, laboratories should endeavour to determine their own cut-offs before relying on baseline LH levels for assessment of precocious puberty.

Timing of post-triptorelin/gonadorelin blood sampling

The peak LH response has been reported to occur at various time points between 30 minutes to 180 minutes post-GnRH/GnRH agonist stimulation. This is dependent on the study design and the GnRH/GnRHa, sampling timepoints, and LH assay used.

If only taking blood samples at baseline and 1–2 timepoints post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, according to the available literature, the best time to take the stimulated LH sample(s) (i.e. the timepoint(s) with the best diagnostic accuracy forCPP) are:

> *Triptorelin studies*: LH sample taken at either 30 minutes, 60 minutes, or 180 minutes post-triptorelin
>
> *Gonadorelin studies*: LH sample taken at either 30 minutes, 40 minutes, 45 minutes, or 60 minutes post-gonadorelin

Please discuss with the consultant responsible for the patient about which timepoints they would like samples to be taken.

Some studies support the additional sampling timepoint of 24 hours post-GnRH/GnRHa stimulation for a testosterone/estradiol level to improve the diagnostic accuracy of the test. Other studies report that this is not required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRHa stimulation testosterone/oestradiol level can also be used in the assessment of delayed puberty. Discuss with the consultant responsible for the patient about whether they would like this 24-hour blood sample taken.

Use and interpretation of GnRH stimulation test in infants and pre-school aged children

Use of the GnRH stimulation test in young children to establish a diagnosis of CPP has its limitations with respect to the interpretation of results. A peak LH >5.0 IU/L is commonly used as the diagnostic cut-off for CPP. However, in infants and pre-school aged children, this peak LH cut-off level is likely too low.

In a Danish study of 48 healthy girls < 6 years of age, assessed clinically to be pre-pubertal, the following LH and FSH responses, measured on the Roche Cobas e601 platform, were achieved at 30 minutes post gonadorelin IV injection (0.1 mg/m^2 body surface area, maximum dose 0.1 mg).

Table 10 Post-gonadorelin LH and FSH responses

	Age group (years)					
	0–1	**1–2**	**2–3**	**3–4**	**4–5**	**5–6**
Stimulated LH (IU/L) Median (minimum, maximum)	7.57 (5.63–7.66)	4.86 (2.38–8.00)	4.31 (2.84–9.96)	2.19 (1.15–3.92)	3.74 (1.63–5.47)	2.61 (0.87–3.46)
Stimulated FSH (IU/L) Median (minimum, maximum)	26.56 (22.82–40.39)	20.51 (16.62–29.43)	20.14 (9.11–36.15)	12.15 (7.94–19.00)	17.22 (10.40–20.69)	11.53 (6.81–26.95)
Stimulated LH/FSH ratio Median (minimum, maximum)	0.21 (0.19–0.33)	0.25 (0.11–0.29)	0.21 (0.14–0.37)	0.16 (0.06–0.37)	0.26 (0.09–0.43)	0.19 (0.07–0.39)

Notes. FSH, follicle stimulating hormone; LH, luteinising hormone

During infancy, usually between 1–6 months of age, there is transient activation of the HPG axis, termed the ‘mini-puberty of infancy’. Performing a GnRH stimulation test duringthe mini-puberty of infancy will generate a positive result.

REFERENCES

See individual protocols.

Section VII

Appendix

GLUCAGON-STIMULATED COPEPTIN TEST

Indication:

To differentiate between arginine vasopressin (AVP) deficiency (due to complete or partial central diabetes insipidus) and primary polydipsia in children who present with polyuria-polydipsia syndrome (PPS).

Of note, an elevated baseline (unstimulated) copeptin level excludes central diabetes insipidus, thus a stimulated copeptin test or water deprivation test is not required and, depending on biochemical and clinical context, AVP resistance (nephrogenic diabetes insipidus) should be considered.

**** Whilst the current literature supports the use of glucagon-stimulated copeptin in differentiating adult patients with PPS, the paediatric reference data remain under development. As such, this protocol should be considered a research tool only, and any copeptin data derived from this test should be interpreted in a research context ****

Rationale:

AVP (also known as anti-diuretic hormone [ADH]) is the principal hormone involved in the regulation of water/fluid balance. It is released from the posterior pituitary gland in response to increasing plasma osmolality and acts on V2 receptors in the kidney. This promotes the reabsorption of water via aquaporin channels, which leads to declining urine volume, increasing urine osmolality, and prevention of further increase in plasma osmolality; thereby maintaining plasma osmolar homeostasis.

After non-AVP related causes have been excluded in children and adolescents with polyuria polydipsia syndrome (PPS), paired morning serum and urinary sodium and osmolality should be measured. If the results are conclusive for AVP deficiency (Na >145 mmol/L with urine osmolality <300 mOsm/kg), no further diagnostic testing is required. However, if the paired samples are inconclusive, assessment of AVP production is indicated. The current gold standard for this is the Water Deprivation Test (WDT). However, the WDT is a distressing and highly burdensome test with limited sensitivity and specificity *(*Fenske 2011; Fenske 2018*)*. More recently, copeptin, which is the C-terminal part of the AVP prohormone, has been identified as an ideal surrogate measure of AVP secretion and activity. Is it released in equimolar amounts to AVP, its plasma half-life is significantly longer, it is more stable, and the assay is far simpler.

Both unstimulated and stimulated copeptin levels are becoming increasingly incorporated into polyuria-polydipsia diagnostic algorithms. Stimulation of copeptin release can be induced by an osmolar (e.g., hypertonic saline) or non-osmolar (e.g., arginine, glucagon) stimulus, but paediatric data are limited. The hypertonic saline-stimulated copeptin test has been shown to be superior in diagnostic accuracy to the WDT in adults (Refardt, 2023) and is considered the new gold standard in differentiating adult patients with PPS, therein replacing the WDT *(*Atila, 2024*)*; however, the hypertonic saline test is not considered safe in the paediatric population. Alternative stimulants of the posterior pituitary, many of which are known stimulants of growth hormone secretion, have been explored in adults and, to a lesser extent, in children; these include arginine, glucagon, insulin, and L-Dopa (Katan, 2007; March, 2023, 2024; Stankovic, 2023;). Whilst arginine is a proven stimulant of copeptin, it was found to be inferior to the hypertonic saline-stimulated copeptin test in adults (Refardt, 2023), and not significantly different to the WDT (Fenske, 2018). In children, arginine was found to be less robust in stimulating copeptin than in adult subjects and inadequate for differentiating partial AVP deficiency from primary polydipsia (Winzeler, 2019; Gippert, 2023).

As an alternative, emerging data in adults suggest glucagon has a strong stimulatory effect on copeptin (Lewandowski, 2016; Atila, 2022). Glucagon is a safe and well-tolerated medication used routinely in children undergoing growth hormone stimulation testing (GHST). The promising data in adults, combined with the reassuring safety profile in children makes glucagon-stimulated copeptin an ideal candidate for the investigation and differentiation of polyuria polydipsia syndrome (PPS) in paediatric patients.

Contraindications:

Intercurrent illness.

Untreated hypothyroidism or hypocortisolism.

Severe renal, cardiac, or liver disease.

Failure to eat for 48 hours or a glycogen storage disorder (GSD). In these patients, glycogen stores are low or cannot be mobilised, which means more marked or unpredictable hypoglycaemia may occur.

It is recommended that potential interactions with medications or supplements concomitantly taken by the patient be checked.

Precautions:

Robust intravenous (IV) access must be attained for repeated blood sampling.

Children younger than 2 years old require very close monitoring during this test. If this cannot be provided in your local day unit, it may be more appropriate to admit the child to hospital and perform the test as an inpatient.

In infants and children younger than 4 years old, and those with untreated growth hormone deficiency (GHD), moderate hypoglycaemia may follow glucagon stimulation testing. Ensure there is readily accessible hypoglycaemia treatment.

Expertise level:

The minimum requirement is for the test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including the ability to site an IV cannula.

Formulation & Dose:

Table 1 Glucagon formulation and dose

Formulation	**Dose**
Glucagon hydrochloride (1mg; powder plus diluent)	30 mcg/kg subcutaneously (maximum 1 mg)

Adverse reactions:

Transient nausea, flushing, vomiting, abdominal pain/cramps, and feelings of apprehension may occur.

Glucagon stimulates a 2–3-fold rise in the blood glucose level following administration. This is maximal within the first hour. Following this rise in blood glucose level and subsequent stimulation of endogenous insulin, *hypo*glycaemia may develop later in the test.

Anaphylaxis is a very rare, but potential, complication

Preparation:

Ensure normal thyroid function and electrolytes prior to commencing the test.

Please ask the consultant responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, on the the request form.

FOOD RESTRICTION - Overnight fast from food (see fasting protocol for age-based maximum fasting durations). In infants, young children, and children prone to hypoglycaemia, the fasting period may be as short as 2 hours.

FLUID RESTRICTION - Water is permitted until 1 hour prior to the commencement of the test.

Equipment:

Equipment/material required for IV cannulation and blood collection –

- IV cannula
- 2-mL and 5-mL syringes
- sodium chloride 0.9% for IV cannula flushes
- blood tubes
- stimulant – glucagon

Method:

1. Ensure the steps from the Preparation section have been undertaken taken prior to proceeding with the test.
2. Weigh the patient, calculate the glucagon dose, and take baseline observations.
3. Insert the IV cannula and collect the copeptin level.
4. Wait 30 minutes post cannulation, then collect baseline (pre-stimulation) blood samples.
5. Administer the glucagon subcutaneously or intramuscularly, as per the dosing table above.
6. Conduct the blood sampling, as outlined below.
7. Check blood glucose levels using a bedside/point-of-care glucometer at each blood sampling timepoint. If the child develops hypoglycaemia during the test, collect a hypoglycaemia screen (if indicated and safe to do so) and then treat the hypoglycaemia as per your local unit's hypoglycaemia management guideline. Consider giving an oral glucose drink if there has been a downward trend and the blood glucose level is <3.2 mmol/L, to help maintain adequate glucose levels. Hypoglycaemia corrected with an oral glucose drink will not compromise the interpretation of the test results. After hypoglycaemia correction, continue with collecting the remaining samples for the stimulation test.
8. No food or drink (other than for the treatment of hypoglycaemia) is permitted until the test is completed.

Sample Collection:

Table 2 Glucagon administration and sampling summary

	-30mins	-1 min	Administer glucagon	60mins	90mins	120mins	150mins	180mins
	Cannula inserted							
Weight	X							X
Vitals (BP, HR)	X	X		X	X	X	X	X
Glucose (+bedside glucose)		X		X	X	X	X	X
Copeptin	X	X		X	X	X	X	X
Serum sodium + osmolality		X		X	X	X	X	X
Urine sodium + osmolality		X						X
Urine time of collection + volume voided								
Other tests as per consultant								

Notes. BP, blood pressure; HR, heart rate

Notes:

Blood tubes/minimum blood volume note

Please confirm with your local laboratory which blood tubes and minimum blood volumes are required to run these tests, as there may be some differences between laboratories.

Minimum volumes are specified for small children and/or those undergoing multiple tests. Please take more blood if this does not apply.

Note: each copeptin level should be collected in its own small volume tube to allow for processing separate to the other bloods collected (ethylenediamine tetraacetic acid [EDTA] and lithium heparin [LiHep] can both be used in the BRAHMS Copeptin proAVP KRYPTOR machine, but the local laboratory preference should be observed).

Interpretation:

There are no paediatric reference data for glucagon-stimulated copeptin. Copeptin results derived from this test should be interpreted with caution.

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