Harmonisation of Endocrine Dynamic Testing in Paediatrics (HDET-Paeds) Protocol Manual

Draft Guidelines

Disclaimer: this draft document is not intended for the purpose of clinical use

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GROWTH HORMONE STIMULATION TEST (GHST)
Stimulant: Arginine

Indications:

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

Rationale:

The hypothalamus stimulates 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.

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), interfere with arginine stimulation

People with 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:

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

Formulation & 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)	Intravenous infusion over 30 minutes

Adverse reactions:

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Preparation:

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

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.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	In the 2-3 days before the day of GH stimulation testing

Sex steroid priming options for males & females

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – arginine

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient, calculate arginine dose and take baseline observations.
3. Insert IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer 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 0.9% saline prior to taking the 30-minute blood sample.
5. Blood sampling as below. If performed as part of a combined pituitary test, see combined protocol.

6. 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.
7. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

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, for example IGF-1, IGFBP-3, ACTH cortisol as per requesting clinician	+/-						
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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 GH stimulation tests. In children who are 5 years and older, GH testing with sex steroid priming is required.	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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.

REFERENCES

1. AbdelNabi R, Al Khalifah R. Growth hormone stimulation test with Clonidine and Arginine an unreported side effect. *J. Clin. Transl. Endocrinol.: Case Rep*. 2020;15. https://doi.org/10.1016/j.jecr.2019.100055
2. Al Khalifah R, Moisan L, Bui H. The shortened combined clonidine and arginine test for growth hormone deficiency is practical and specific: a diagnostic accuracy study. *J Pediatr Endocrinol Metab*. 2016;29(3):305-310. doi:10.1515/jpem-2015-0284
3. Australia and New Zealand Society for Paediatric Endocrinology and Diabetes (ANZSPED) website: Clinical Resources & Links > Growth & Growth Charts. https://anzsped.org/clinical-resources-links/growth-growth-charts/
4. Barrett J, Maranda L, Nwosu BU. The Relationship between Subnormal Peak-Stimulated Growth Hormone Levels and Auxological Characteristics in Obese Children. *Front Endocrinol (Lausanne)*. 2014;5:35. Published 2014 Mar 25. doi:10.3389/fendo.2014.00035
5. Bhat N, Dulmovits E, Lane A, Messina C, Wilson T. Combined simultaneous arginine clonidine stimulation test: Timing of peak growth hormone (GH) concentration and correlation with clinical indices of GH status. *Growth Horm IGF Res*. 2018;40:28-31. doi:10.1016/j.ghir.2018.04.002
6. Binder G, Reinehr T, Ibáñez L, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr*. 2019;92(3):150-156. doi:10.1159/000503783
7. Bizzarri C, Pedicelli S, Boscherini B, Bedogni G, Cappa M, Cianfarani S. Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency. *J Endocrinol Invest*. 2015;38(4):429-436. doi:10.1007/s40618-014-0205-3
8. Chesover AD, Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clin Endocrinol (Oxf)*. 2016;84(5):708-714. doi:10.1111/cen.13035
9. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):737-747. doi:10.1016/j.beem.2016.11.002
10. Dori EB, Avnon Ziv C, Auerbach A, Greenberg Y, Zaken H, Levy-Khademi F. The inter - Test variability of growth hormone stimulation tests and factors affecting this variability. *Growth Horm IGF Res*. 2020;55:101361. doi:10.1016/j.ghir.2020.101361
11. Duncan G, Kiff S, Mitchell RT. Sex steroid priming for growth hormone stimulation testing in children and adolescents with short stature: A systematic review. *Clin Endocrinol (Oxf)*. 2023;98(4):527-535. doi:10.1111/cen.14862
12. Frerichs C, Raymond L, Senniappan S. Variations in sex steroid priming for growth hormone stimulation testing in UK. *Arch Dis Child*. 2017;102(3):294. doi:10.1136/archdischild-2016-311186
13. Gabreanu GR. An update on the diagnosis of growth hormone deficiency. *Discoveries (Craiova)*. 2018;6(1):e82. Published 2018 Apr 12. doi:10.15190/d.2018.2
14. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;85(11):3990-3993. doi:10.1210/jcem.85.11.6984

15. Gillis D, Granat N, Strich D. The arginine stimulation test: timing of peak is not a helpful parameter in the diagnosis of growth hormone deficiency. *J Pediatr Endocrinol Metab*. 2013;26(9-10):813-817. doi:10.1515/jpem-2013-0075
16. Glibbery M, Fleming A, Chanchlani R, et al. Myalgia and Hematuria in Association with Clonidine and Arginine Administration for Growth Hormone Stimulation Tests. *Case Rep Med*. 2020;2020:4827072. Published 2020 May 26. doi:10.1155/2020/4827072
17. Kim JH, Chae HW, Chin SO, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement from Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab (Seoul)*. 2020;35(2):272-287. doi:10.3803/EnM.2020.35.2.272
18. Lee NY, Kim SE, Kim S, et al. Effect of body mass index on peak growth hormone level after growth hormone stimulation test in children with short stature. *Ann Pediatr Endocrinol Metab*. 2021;26(3):192-198. doi:10.6065/apem.2040246.123
19. Lee HS, Hwang JS. Influence of body mass index on growth hormone responses to classic provocative tests in children with short stature. *Neuroendocrinology*. 2011;93(4):259-264. doi:10.1159/000326838
20. Lennartsson O, Nilsson O, Lodefalk M. Discordance Between Stimulated and Spontaneous Growth Hormone Levels in Short Children Is Dependent on Cut-Off Level and Partly Explained by Refractoriness. *Front Endocrinol (Lausanne)*. 2020;11:584906. Published 2020 Nov 17. doi:10.3389/fendo.2020.584906
21. Marinkovic M, Newfield RS. Self-limiting hematuria following growth hormone provocative testing with arginine hydrochloride. *J Pediatr Endocrinol Metab*. 2012;25(7-8):791-793. doi:10.1515/jpem-2012-0160
22. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85(11):4168-4172. doi:10.1210/jcem.85.11.6928
23. Mauras N, Walton P, Nicar M, Welch S, Rogol AD. Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*. 2000;48(5):614-618. doi:10.1203/00006450-200011000-00010
24. Muster L., Zangen D, Nesher R, et al. Arginine and Clonidine Stimulation Tests for Growth Hormone Deficiency Revisited - Do We Really Need So Many Samples?. *Journal of Pediatric Endocrinology and Metabolism*, 2009;*22*(3): 215-224. https://doi.org/10.1515/JPEM.2009.22.3.215
25. Nwosu BU, Coco M, Jones J, Barnes KM, Yanovski JA, Baron J. Short stature with normal growth hormone stimulation testing: lack of evidence for partial growth hormone deficiency or insensitivity. *Horm Res*. 2004;62(2):97-102. doi:10.1159/000079711
26. Pharmac Special Authority for Somatropin (SA 2032). https://schedule.pharmac.govt.nz/latest/SA2032.pdf
27. Richmond E, Rogol AD. Testing for growth hormone deficiency in children. *Growth Horm IGF Res*. 2020;50:57-60. doi:10.1016/j.ghir.2019.12.002
28. Rochiccioli P, Enjaume C, Tauber MT, Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatr*. 1993;82(3):245-248. doi:10.1111/j.1651-2227.1993.tb12652.x

29. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre- and early adolescent children is evidence based. *Horm Res Paediatr*. 2011;75(1):78-80. doi:10.1159/000323353
30. Snyder C, Hess C. Abstract: Tissue Necrosis: A Rare Adverse Event of Arginine Infusion during Growth Hormone Stimulation Testing. *J Pediatr Nurs.* 2016;31(3):364.
31. Sodero G, Mariani F, Caprarelli M, et al. Growth hormone responses during arginine and clonidine stimulation test: Correlations with patients' auxological and metabolic parameters in a single centre study. *Growth Horm IGF Res*. 2023;68:101522. doi:10.1016/j.ghir.2022.101522
32. Stanley T. Diagnosis of growth hormone deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(1):47-52. doi:10.1097/MED.0b013e32834ec952
33. Thieme F, Vogel M, Gausche R, et al. The Influence of Body Mass Index on the Growth Hormone Peak Response regarding Growth Hormone Stimulation Tests in Children. *Horm Res Paediatr*. 2022;95(5):452-460. doi:10.1159/000526240
34. Thirunagari R, Marrone A, Elsinghorst H, Mastrandrea LD. Hematuria as an adverse outcome following provocative growth hormone stimulation testing in children. *J Pediatr Endocrinol Metab*. 2018;31(5):539-543. doi:10.1515/jpem-2017-0458
35. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1049-1055. doi:10.1515/jpem.2011.327
36. Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M, Oron T. Diagnosis of Growth Hormone Deficiency in Children: The Efficacy of Glucagon versus Clonidine Stimulation Test. *Horm Res Paediatr*. 2020;93(7-8):470-476. doi:10.1159/000513393
37. Yau M, Chacko E, Regelmann MO, et al. Peak Growth Hormone Response to Combined Stimulation Test in 315 Children and Correlations with Metabolic Parameters. *Horm Res Paediatr*. 2019;92(1):36-44. doi:10.1159/000502308
38. Yau M, Rapaport R. Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. *Front Endocrinol (Lausanne)*. 2022;13:902364. Published 2022 Jun 9. doi:10.3389/fendo.2022.902364
39. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. *Endocr Connect*. 2023;12(7):e220504. Published 2023 Jun 12. doi:10.1530/EC-22-0504

GROWTH HORMONE STIMULATION TEST (GHST)
Stimulant: Glucagon

Indications:

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

2. To test adrenocorticotropic hormone (ACTH) release from the anterior pituitary in individuals being assessed for ACTH/cortisol deficiency. Please note, glucagon stimulation of the hypothalamic-pituitary-adrenal axis is not robust and, therefore, an inadequate cortisol response should not be interpreted in isolation as adrenal insufficiency.

Rationale:

The hypothalamus stimulates 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.

The hypothalamus stimulates release of ACTH from corticotrophs in the anterior pituitary gland via corticotropin-releasing hormone (CRH). ACTH then acts on the adrenal cortex to stimulate production and secretion of cortisol. Evaluation of this response is important in the evaluation of disorders of the hypothalamic-pituitary-adrenal axis.

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 48hours, 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.

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:

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

Formulation & Dose:

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

Adverse reactions:

Transient nausea, flushing, vomiting for 1 – 2 minutes, abdominal pain / cramps, 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 patient is euthyroid and has normal TFTs prior to commencing test.

Overnight fast. 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.

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.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	Dose	Duration
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	In the 2-3 days before the day of GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – glucagon

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient, calculate glucagon dose and take baseline observations.
3. Insert IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer glucagon subcutaneously or intramuscularly as per the dosing table above.
5. Blood sampling as below. If performed as part of a combined pituitary test, see combined protocol.
6. 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. Consider giving an oral glucose drink if BGL < 3.2 mmol/L to help maintain adequate glucose levels. Hypoglycaemia corrected with an oral glucose drink will not compromise interpretation of the test results.
7. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered:		**Dose Administered:**			**Time Administered:**		
	Minutes pre-glucagon	**Administer glucagon**	**Minutes post-glucagon**				
Actual time bloods taken:							
Test	-1 Min		60 Min	90 Min	120 Min	150 Min	180 Min

GH	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓
Cortisol	✓		✓	✓	✓	✓	✓
Other tests e.g. IGF-1, IGFBP-3 as per requesting clinician	+/-						
Sample Tubes / Minimum Blood Volume							

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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 GH stimulation tests. In children who are 5 years and older, GH testing with sex steroid priming is required.	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

See short synacthen test protocol for interpretation of cortisol levels.

Please note that the specificity of the glucagon stimulation test 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.

REFERENCES

1. Al Balwi R, Al-Qahtani M, Alrowished AK, et al. Reliability of Agreement between Insulin, Clonidine, and Glucagon Stimulation Tests for the Diagnosis of Growth Hormone Deficiency in Children: A Retrospective Cohort Study. *Children (Basel)*. 2023;10(8):1381. doi:10.3390/children10081381
2. Binder G, Reinehr T, Ibáñez L, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr*. 2019;92(3):150-156. doi:10.1159/000503783
3. Bizzarri C, Pedicelli S, Boscherini B, Bedogni G, Cappa M, Cianfarani S. Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency. *J Endocrinol Invest*. 2015;38(4):429-436. doi:10.1007/s40618-014-0205-3
4. Chesover AD, Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clin Endocrinol (Oxf)*. 2016;84(5):708-714. doi:10.1111/cen.13035
5. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):737-747. doi:10.1016/j.beem.2016.11.002
6. Christoforidis A, Triantafyllou P, Slavakis A, Katzos G. Clonidine and glucagon stimulation for testing growth hormone secretion in children and adolescents: can we make it with fewer samples?. *J Endocrinol Invest*. 2013;36(11):1046-1050. doi:10.3275/9061
7. Dori EB, Avnon Ziv C, Auerbach A, Greenberg Y, Zaken H, Levy-Khademi F. The inter - Test variability of growth hormone stimulation tests and factors affecting this variability. *Growth Horm IGF Res*. 2020;55:101361. doi:10.1016/j.ghir.2020.101361
8. Duncan G, Kiff S, Mitchell RT. Sex steroid priming for growth hormone stimulation testing in children and adolescents with short stature: A systematic review. *Clin Endocrinol (Oxf)*. 2023;98(4):527-535. doi:10.1111/cen.14862
9. Frerichs C, Raymond L, Senniappan S. Variations in sex steroid priming for growth hormone stimulation testing in UK. *Arch Dis Child*. 2017;102(3):294. doi:10.1136/archdischild-2016-311186
10. Gabreanu GR. An update on the diagnosis of growth hormone deficiency. *Discoveries (Craiova)*. 2018;6(1):e82. Published 2018 Apr 12. doi:10.15190/d.2018.2
11. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;85(11):3990-3993. doi:10.1210/jcem.85.11.6984
12. Kim JH, Chae HW, Chin SO, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement from Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab (Seoul)*. 2020;35(2):272-287. doi:10.3803/EnM.2020.35.2.272
13. Lee NY, Kim SE, Kim S, et al. Effect of body mass index on peak growth hormone level after growth hormone stimulation test in children with short stature. *Ann Pediatr Endocrinol Metab*. 2021;26(3):192-198. doi:10.6065/apem.2040246.123
14. Lee HS, Hwang JS. Influence of body mass index on growth hormone responses to classic provocative tests in children with short stature. *Neuroendocrinology*. 2011;93(4):259-264. doi:10.1159/000326838

15. Lennartsson O, Nilsson O, Lodefalk M. Discordance Between Stimulated and Spontaneous Growth Hormone Levels in Short Children Is Dependent on Cut-Off Level and Partly Explained by Refractoriness. *Front Endocrinol (Lausanne)*. 2020;11:584906. Published 2020 Nov 17. doi:10.3389/fendo.2020.584906
16. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85(11):4168-4172. doi:10.1210/jcem.85.11.6928
17. Mauras N, Walton P, Nicar M, Welch S, Rogol AD. Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*. 2000;48(5):614-618. doi:10.1203/00006450-200011000-00010
18. Nwosu BU, Coco M, Jones J, Barnes KM, Yanovski JA, Baron J. Short stature with normal growth hormone stimulation testing: lack of evidence for partial growth hormone deficiency or insensitivity. *Horm Res*. 2004;62(2):97-102. doi:10.1159/000079711
19. Rameez Raja B, Natarajan V, Sridhar S. Abstract 87: A comparative study to validate peak growth hormone levels in clonidine and glucagon stimulation test in severe short stature. *Indian J. Endocrinol. Metab.* 2022;26(Suppl 8):S36-S37. doi:10.4103/2230-8210.363778.
20. Richmond E, Rogol AD. Testing for growth hormone deficiency in children. *Growth Horm IGF Res*. 2020;50:57-60. doi:10.1016/j.ghir.2019.12.002
21. Rochiccioli P, Enjaume C, Tauber MT, Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatr*. 1993;82(3):245-248. doi:10.1111/j.1651-2227.1993.tb12652.x
22. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre- and early adolescent children is evidence based. *Horm Res Paediatr*. 2011;75(1):78-80. doi:10.1159/000323353
23. Secco A, di Iorgi N, Napoli F, et al. The glucagon test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. *J Clin Endocrinol Metab*. 2009;94(11):4251-4257. doi:10.1210/jc.2009-0779
24. Stanley T. Diagnosis of growth hormone deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(1):47-52. doi:10.1097/MED.0b013e32834ec952
25. Strich D, Terespolsky N, Gillis D. Glucagon stimulation test for childhood growth hormone deficiency: timing of the peak is important. *J Pediatr*. 2009;154(3):415-419. doi:10.1016/j.jpeds.2008.08.044
26. Thieme F, Vogel M, Gausche R, et al. The Influence of Body Mass Index on the Growth Hormone Peak Response regarding Growth Hormone Stimulation Tests in Children. *Horm Res Paediatr*. 2022;95(5):452-460. doi:10.1159/000526240
27. Thirunagari R, Marrone A, Elsinghorst H, Mastrandrea LD. Hematuria as an adverse outcome following provocative growth hormone stimulation testing in children. *J Pediatr Endocrinol Metab*. 2018;31(5):539-543. doi:10.1515/jpem-2017-0458
28. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1049-1055. doi:10.1515/jpem.2011.327

29. Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M, Oron T. Diagnosis of Growth Hormone Deficiency in Children: The Efficacy of Glucagon versus Clonidine Stimulation Test. *Horm Res Paediatr*. 2020;93(7-8):470-476. doi:10.1159/000513393
30. Yau M, Rapaport R. Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. *Front Endocrinol (Lausanne)*. 2022;13:902364. Published 2022 Jun 9. doi:10.3389/fendo.2022.902364
31. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. *Endocr Connect*. 2023;12(7):e220504. Published 2023 Jun 12. doi:10.1530/EC-22-0504

GROWTH HORMONE STIMULATION TEST
Stimulant: Clonidine

Indications:

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

Rationale:

The hypothalamus stimulates 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.

Contraindications:

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

Untreated adrenal insufficiency, hypothyroidism, panhypopituitarism

Caution in children with known congenital / acquired heart disease

Expertise level:

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

Formulation & 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 PBS, Australia

Clonidine 25 microgram and 150 microgram tablets available in New Zealand

Adverse reactions:

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

Hypotension, postural hypotension. Fall in blood pressure by ~10 mmHg about 1 hour after ingestion. Usually resolves by the end of the test but may last several hours. Effect 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 patient is euthyroid and has normal TFTs prior to commencing test.

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

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.

Overnight fast. Water is permitted.

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

Sex steroid priming

It is recommended that sex steroid priming is used in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	In the 2-3 days before the day of GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – clonidine

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Ideally perform test first thing in the morning following an overnight fast. However, minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.

2. Weigh patient, calculate clonidine dose and take baseline observations.
3. Ensure child is recumbent and resting during the test. Can drink water during the test. No food until test completed.
4. Insert IV cannula and take baseline (pre-stimulation) blood samples. Flush IV cannula with 0.9% sodium chloride.
5. Administer clonidine orally (with water) as per the dosing table above.
6. Give 10 ml/kg IV bolus of 0.9% sodium chloride over 30 minutes following clonidine administration to minimise the fall in blood pressure. **The clinician may choose to give a volume less than 10 ml/kg depending on the size/age of the child.
7. Timing of further blood sampling as per table below. If performed as part of a combined pituitary test, see combined protocol.
8. Check a 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.
9. 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 chlordie bolus. If unsure or no response, call medical team for advice.
10. Take care ambulating the child following completion of the test. Postural hypotension may occur.
11. No food until the test is completed. Water is permitted.

Discharge:

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 abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

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, IGFBP2 as per requesting clinician							
Sample Tubes / Minimum Blood Volume							

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH deficient sequelae (for	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an

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.	adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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.

REFERENCES

1. AbdelNabi R, Al Khalifah R. Growth hormone stimulation test with Clonidine and Arginine an unreported side effect. *J. Clin. Transl. Endocrinol.* 2020:15. doi.org/10.1016/j.jecr.2019.100055
2. Al Balwi R, Al-Qahtani M, Alrowished AK, et al. Reliability of Agreement between Insulin, Clonidine, and Glucagon Stimulation Tests for the Diagnosis of Growth Hormone Deficiency in Children: A Retrospective Cohort Study. *Children (Basel)*. 2023;10(8):1381. doi:10.3390/children10081381
3. Al Khalifah R, Moisan L, Bui H. The shortened combined clonidine and arginine test for growth hormone deficiency is practical and specific: a diagnostic accuracy study. *J Pediatr Endocrinol Metab*. 2016;29(3):305-310. doi:10.1515/jpem-2015-0284
4. Australia and New Zealand Society for Paediatric Endocrinology and Diabetes (ANZSPED) website: Clinical Resources & Links > Growth & Growth Charts. https://anzsped.org/clinical-resources-links/growth-growth-charts/
5. Barrett J, Maranda L, Nwosu BU. The Relationship between Subnormal Peak-Stimulated Growth Hormone Levels and Auxological Characteristics in Obese Children. *Front Endocrinol (Lausanne)*. 2014;5:35. Published 2014 Mar 25. doi:10.3389/fendo.2014.00035
6. Bhat N, Dulmovits E, Lane A, Messina C, Wilson T. Combined simultaneous arginine clonidine stimulation test: Timing of peak growth hormone (GH) concentration and correlation with clinical indices of GH status. *Growth Horm IGF Res*. 2018;40:28-31. doi:10.1016/j.ghir.2018.04.002
7. Binder G, Reinehr T, Ibáñez L, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr*. 2019;92(3):150-156. doi:10.1159/000503783
8. Bizzarri C, Pedicelli S, Boscherini B, Bedogni G, Cappa M, Cianfarani S. Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency. *J Endocrinol Invest*. 2015;38(4):429-436. doi:10.1007/s40618-014-0205-3
9. Chesover AD, Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clin Endocrinol (Oxf)*. 2016;84(5):708-714. doi:10.1111/cen.13035
10. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):737-747. doi:10.1016/j.beem.2016.11.002
11. Christoforidis A, Triantafyllou P, Slavakis A, Katzos G. Clonidine and glucagon stimulation for testing growth hormone secretion in children and adolescents: can we make it with fewer samples?. *J Endocrinol Invest*. 2013;36(11):1046-1050. doi:10.3275/9061
12. Dori EB, Avnon Ziv C, Auerbach A, Greenberg Y, Zaken H, Levy-Khademi F. The inter - Test variability of growth hormone stimulation tests and factors affecting this variability. *Growth Horm IGF Res*. 2020;55:101361. doi:10.1016/j.ghir.2020.101361
13. Duncan G, Kiff S, Mitchell RT. Sex steroid priming for growth hormone stimulation testing in children and adolescents with short stature: A systematic review. *Clin Endocrinol (Oxf)*. 2023;98(4):527-535. doi:10.1111/cen.14862
14. Frerichs C, Raymond L, Senniappan S. Variations in sex steroid priming for growth hormone stimulation

testing in UK. *Arch Dis Child*. 2017;102(3):294. doi:10.1136/archdischild-2016-311186

15. Gabreanu GR. An update on the diagnosis of growth hormone deficiency. *Discoveries (Craiova)*. 2018;6(1):e82. Published 2018 Apr 12. doi:10.15190/d.2018.2
16. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;85(11):3990-3993. doi:10.1210/jcem.85.11.6984
17. Glibbery M, Fleming A, Chanchlani R, et al. Myalgia and Hematuria in Association with Clonidine and Arginine Administration for Growth Hormone Stimulation Tests. *Case Rep Med*. 2020;2020:4827072. Published 2020 May 26. doi:10.1155/2020/4827072
18. Kim JH, Chae HW, Chin SO, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement from Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab (Seoul)*. 2020;35(2):272-287. doi:10.3803/EnM.2020.35.2.272
19. Lee NY, Kim SE, Kim S, et al. Effect of body mass index on peak growth hormone level after growth hormone stimulation test in children with short stature. *Ann Pediatr Endocrinol Metab*. 2021;26(3):192-198. doi:10.6065/apem.2040246.123
20. Lee HS, Hwang JS. Influence of body mass index on growth hormone responses to classic provocative tests in children with short stature. *Neuroendocrinology*. 2011;93(4):259-264. doi:10.1159/000326838
21. Lennartsson O, Nilsson O, Lodefalk M. Discordance Between Stimulated and Spontaneous Growth Hormone Levels in Short Children Is Dependent on Cut-Off Level and Partly Explained by Refractoriness. *Front Endocrinol (Lausanne)*. 2020;11:584906. Published 2020 Nov 17. doi:10.3389/fendo.2020.584906
22. Loche S, Guzzetti C, Pilia S, et al. Effect of body mass index on the growth hormone response to clonidine stimulation testing in children with short stature. *Clin Endocrinol (Oxf)*. 2011;74(6):726-731. doi:10.1111/j.1365-2265.2011.03988.x
23. Marinkovic M, Newfield RS. Self-limiting hematuria following growth hormone provocative testing with arginine hydrochloride. *J Pediatr Endocrinol Metab*. 2012;25(7-8):791-793. doi:10.1515/jpem-2012-0160
24. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85(11):4168-4172. doi:10.1210/jcem.85.11.6928
25. Mauras N, Walton P, Nicar M, Welch S, Rogol AD. Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*. 2000;48(5):614-618. doi:10.1203/00006450-200011000-00010
26. Morris AH, Harrington MH, Churchill DL, Olshan JS. Growth hormone stimulation testing with oral clonidine: 90 minutes is the preferred duration for the assessment of growth hormone reserve. *J Pediatr Endocrinol Metab*. 2001;14(9):1657-1660. doi:10.1515/jpem.2001.14.9.1657
27. Muster L, Zangen DH, Nesher R, Hirsch HJ, Muster Z, Gillis D. Arginine and clonidine stimulation tests for growth hormone deficiency revisited--do we really need so many samples?. *J Pediatr Endocrinol Metab*. 2009;22(3):215-223. doi:10.1515/jpem.2009.22.3.215

28. Nwosu BU, Coco M, Jones J, Barnes KM, Yanovski JA, Baron J. Short stature with normal growth hormone stimulation testing: lack of evidence for partial growth hormone deficiency or insensitivity. *Horm Res*. 2004;62(2):97-102. doi:10.1159/000079711
29. Pharmac Special Authority for Somatropin (SA 2032). https://schedule.pharmac.govt.nz/latest/SA2032.pdf
30. Rameez Raja B, Natarajan V, Sridhar S. Abstract 87: A comparative study to validate peak growth hormone levels in clonidine and glucagon stimulation test in severe short stature. *Indian J. Endocrinol. Metab.* 2022;26(Suppl 8):S36-S37. doi:10.4103/2230-8210.363778.
31. Richmond E, Rogol AD. Testing for growth hormone deficiency in children. *Growth Horm IGF Res*. 2020;50:57-60. doi:10.1016/j.ghir.2019.12.002
32. Rochiccioli P, Enjaume C, Tauber MT, Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatr*. 1993;82(3):245-248. doi:10.1111/j.1651-2227.1993.tb12652.x
33. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre- and early adolescent children is evidence based. *Horm Res Paediatr*. 2011;75(1):78-80. doi:10.1159/000323353
34. Sodero G, Mariani F, Caprarelli M, et al. Growth hormone responses during arginine and clonidine stimulation test: Correlations with patients' auxological and metabolic parameters in a single centre study. *Growth Horm IGF Res*. 2023;68:101522. doi:10.1016/j.ghir.2022.101522
35. Stanley T. Diagnosis of growth hormone deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(1):47-52. doi:10.1097/MED.0b013e32834ec952
36. Thakur DS, Bhagwat NM, Bhide MM, et al. Clonidine Stimulation Test: Is Single Best Time Point, Convenient Yet Efficacious?. *Indian J Endocrinol Metab*. 2018;22(4):511-514. doi:10.4103/ijem.IJEM_101_18
37. Thieme F, Vogel M, Gausche R, et al. The Influence of Body Mass Index on the Growth Hormone Peak Response regarding Growth Hormone Stimulation Tests in Children. *Horm Res Paediatr*. 2022;95(5):452-460. doi:10.1159/000526240
38. Thirunagari R, Marrone A, Elsinghorst H, Mastrandrea LD. Hematuria as an adverse outcome following provocative growth hormone stimulation testing in children. *J Pediatr Endocrinol Metab*. 2018;31(5):539-543. doi:10.1515/jpem-2017-0458
39. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1049-1055. doi:10.1515/jpem.2011.327
40. Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M, Oron T. Diagnosis of Growth Hormone Deficiency in Children: The Efficacy of Glucagon versus Clonidine Stimulation Test. *Horm Res Paediatr*. 2020;93(7-8):470-476. doi:10.1159/000513393
41. Yau M, Rapaport R. Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. *Front Endocrinol (Lausanne)*. 2022;13:902364. Published 2022 Jun 9. doi:10.3389/fendo.2022.902364
42. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. *Endocr*

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GROWTH HORMONE STIMULATION TEST
Combined Protocol
Stimulants: Arginine and Glucagon

Indications:

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

2. To test adrenocorticotropic hormone (ACTH) release from the anterior pituitary in individuals being assessed for ACTH/cortisol deficiency. Please note, glucagon stimulation of the hypothalamic-pituitary-adrenal axis is not robust and, therefore, an inadequate cortisol response should not be interpreted in isolation as adrenal insufficiency.

Rationale:

The hypothalamus stimulates 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.

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 48hours, 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 (glucagon stimulation test is unreliable in individuals with diabetes as this GH ‘stimulus’ requires endogenous insulin)

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

People with 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 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:

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

Formulation & 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)	Intravenous infusion over 30 minutes

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

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Glucagon

Transient nausea, flushing, vomiting for 1 – 2 minutes, abdominal pain / cramps, 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 patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

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.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	In the 2-3 days before the day of GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, glucagon

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient, calculate arginine and glucagon doses and take baseline observations.
3. Insert IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer arginine via intravenous infusion over 30 minutes. The time that the infusion STARTS (not finishes) is Time 0. Allow time to give a 10 – 15 ml flush with 0.9% saline prior to taking the 30 minute blood sample.
5. Administer glucagon subcutaneously or intramuscularly (dose as per dosing table above) as soon as+90Min blood sample has been collected.
6. Blood sampling as per table below.
7. 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.
8. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered				**Dose Administered**						**Time Administered**				
	Baseline		Minutes post START of arginine infusion											
Actual time bloods taken		Administer arginine						Administer glucagon						
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		150 Min	180 Min	210 Min	240 Min	270 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Cortisol									✓	✓	✓	✓	✓
Other tests e.g. IGF-1, IGFBP-3, ACTH cortisol as per requesting clinician	+/-												
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL						

Interpretation:

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH deficient sequelae (e.g. 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	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test.

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.	Patients with isolated GHD require 2 GHST, 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.

See short synacthen test protocol for interpretation of cortisol levels.

Please note that the specificity of the glucagon stimulation test 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.

REFERENCES

1. AbdelNabi R, Al Khalifah R. Growth hormone stimulation test with Clonidine and Arginine an unreported side effect. *J. Clin. Transl. Endocrinol.: Case Rep*. 2020;15. https://doi.org/10.1016/j.jecr.2019.100055
2. Al Balwi R, Al-Qahtani M, Alrowished AK, et al. Reliability of Agreement between Insulin, Clonidine, and Glucagon Stimulation Tests for the Diagnosis of Growth Hormone Deficiency in Children: A Retrospective Cohort Study. *Children (Basel)*. 2023;10(8):1381. doi:10.3390/children10081381
3. Al Khalifah R, Moisan L, Bui H. The shortened combined clonidine and arginine test for growth hormone deficiency is practical and specific: a diagnostic accuracy study. *J Pediatr Endocrinol Metab*. 2016;29(3):305-310. doi:10.1515/jpem-2015-0284
4. Australia and New Zealand Society for Paediatric Endocrinology and Diabetes (ANZSPED) website: Clinical Resources & Links > Growth & Growth Charts. https://anzsped.org/clinical-resources-links/growth-growth-charts/
5. Barrett J, Maranda L, Nwosu BU. The Relationship between Subnormal Peak-Stimulated Growth Hormone Levels and Auxological Characteristics in Obese Children. *Front Endocrinol (Lausanne)*. 2014;5:35. Published 2014 Mar 25. doi:10.3389/fendo.2014.00035
6. Bhat N, Dulmovits E, Lane A, Messina C, Wilson T. Combined simultaneous arginine clonidine stimulation test: Timing of peak growth hormone (GH) concentration and correlation with clinical indices of GH status. *Growth Horm IGF Res*. 2018;40:28-31. doi:10.1016/j.ghir.2018.04.002
7. Binder G, Reinehr T, Ibáñez L, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr*. 2019;92(3):150-156. doi:10.1159/000503783
8. Bizzarri C, Pedicelli S, Boscherini B, Bedogni G, Cappa M, Cianfarani S. Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency. *J Endocrinol Invest*. 2015;38(4):429-436. doi:10.1007/s40618-014-0205-3
9. Chesover AD, Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clin Endocrinol (Oxf)*. 2016;84(5):708-714. doi:10.1111/cen.13035
10. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):737-747. doi:10.1016/j.beem.2016.11.002
11. Christoforidis A, Triantafyllou P, Slavakis A, Katzos G. Clonidine and glucagon stimulation for testing growth hormone secretion in children and adolescents: can we make it with fewer samples?. *J Endocrinol Invest*. 2013;36(11):1046-1050. doi:10.3275/9061
12. Dori EB, Avnon Ziv C, Auerbach A, Greenberg Y, Zaken H, Levy-Khademi F. The inter - Test variability of growth hormone stimulation tests and factors affecting this variability. *Growth Horm IGF Res*. 2020;55:101361. doi:10.1016/j.ghir.2020.101361
13. Duncan G, Kiff S, Mitchell RT. Sex steroid priming for growth hormone stimulation testing in children and adolescents with short stature: A systematic review. *Clin Endocrinol (Oxf)*. 2023;98(4):527-535. doi:10.1111/cen.14862

14. Frerichs C, Raymond L, Senniappan S. Variations in sex steroid priming for growth hormone stimulation testing in UK. *Arch Dis Child*. 2017;102(3):294. doi:10.1136/archdischild-2016-311186
15. Gabreanu GR. An update on the diagnosis of growth hormone deficiency. *Discoveries (Craiova)*. 2018;6(1):e82. Published 2018 Apr 12. doi:10.15190/d.2018.2
16. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;85(11):3990-3993. doi:10.1210/jcem.85.11.6984
17. Gillis D, Granat N, Strich D. The arginine stimulation test: timing of peak is not a helpful parameter in the diagnosis of growth hormone deficiency. *J Pediatr Endocrinol Metab*. 2013;26(9-10):813-817. doi:10.1515/jpem-2013-0075
18. Glibbery M, Fleming A, Chanchlani R, et al. Myalgia and Hematuria in Association with Clonidine and Arginine Administration for Growth Hormone Stimulation Tests. *Case Rep Med*. 2020;2020:4827072. Published 2020 May 26. doi:10.1155/2020/4827072
19. Kim JH, Chae HW, Chin SO, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement from Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab (Seoul)*. 2020;35(2):272-287. doi:10.3803/EnM.2020.35.2.272
20. Lee NY, Kim SE, Kim S, et al. Effect of body mass index on peak growth hormone level after growth hormone stimulation test in children with short stature. *Ann Pediatr Endocrinol Metab*. 2021;26(3):192-198. doi:10.6065/apem.2040246.123
21. Lee HS, Hwang JS. Influence of body mass index on growth hormone responses to classic provocative tests in children with short stature. *Neuroendocrinology*. 2011;93(4):259-264. doi:10.1159/000326838
22. Lennartsson O, Nilsson O, Lodefalk M. Discordance Between Stimulated and Spontaneous Growth Hormone Levels in Short Children Is Dependent on Cut-Off Level and Partly Explained by Refractoriness. *Front Endocrinol (Lausanne)*. 2020;11:584906. Published 2020 Nov 17. doi:10.3389/fendo.2020.584906
23. Marinkovic M, Newfield RS. Self-limiting hematuria following growth hormone provocative testing with arginine hydrochloride. *J Pediatr Endocrinol Metab*. 2012;25(7-8):791-793. doi:10.1515/jpem-2012-0160
24. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85(11):4168-4172. doi:10.1210/jcem.85.11.6928
25. Mauras N, Walton P, Nicar M, Welch S, Rogol AD. Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*. 2000;48(5):614-618. doi:10.1203/00006450-200011000-00010
26. Muster L., Zangen D, Nesher R, et al. Arginine and Clonidine Stimulation Tests for Growth Hormone Deficiency Revisited - Do We Really Need So Many Samples?. *Journal of Pediatric Endocrinology and Metabolism*, 2009;*22*(3): 215-224. https://doi.org/10.1515/JPEM.2009.22.3.215

27. Nwosu BU, Coco M, Jones J, Barnes KM, Yanovski JA, Baron J. Short stature with normal growth hormone stimulation testing: lack of evidence for partial growth hormone deficiency or insensitivity. *Horm Res*. 2004;62(2):97-102. doi:10.1159/000079711
28. Pharmac Special Authority for Somatropin (SA 2032). https://schedule.pharmac.govt.nz/latest/SA2032.pdf
29. Rameez Raja B, Natarajan V, Sridhar S. Abstract 87: A comparative study to validate peak growth hormone levels in clonidine and glucagon stimulation test in severe short stature. *Indian J. Endocrinol. Metab.* 2022;26(Suppl 8):S36-S37. doi:10.4103/2230-8210.363778.
30. Richmond E, Rogol AD. Testing for growth hormone deficiency in children. *Growth Horm IGF Res*. 2020;50:57-60. doi:10.1016/j.ghir.2019.12.002
31. Rochiccioli P, Enjaume C, Tauber MT, Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatr*. 1993;82(3):245-248. doi:10.1111/j.1651-2227.1993.tb12652.x
32. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre- and early adolescent children is evidence based. *Horm Res Paediatr*. 2011;75(1):78-80. doi:10.1159/000323353
33. Secco A, di Iorgi N, Napoli F, et al. The glucagon test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. *J Clin Endocrinol Metab*. 2009;94(11):4251-4257. doi:10.1210/jc.2009-0779
34. Snyder C, Hess C. Abstract: Tissue Necrosis: A Rare Adverse Event of Arginine Infusion during Growth Hormone Stimulation Testing. *J Pediatr Nurs.* 2016;31(3):364.
35. Sodero G, Mariani F, Caprarelli M, et al. Growth hormone responses during arginine and clonidine stimulation test: Correlations with patients' auxological and metabolic parameters in a single centre study. *Growth Horm IGF Res*. 2023;68:101522. doi:10.1016/j.ghir.2022.101522
36. Stanley T. Diagnosis of growth hormone deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(1):47-52. doi:10.1097/MED.0b013e32834ec952
37. Strich D, Terespolsky N, Gillis D. Glucagon stimulation test for childhood growth hormone deficiency: timing of the peak is important. *J Pediatr*. 2009;154(3):415-419. doi:10.1016/j.jpeds.2008.08.044
38. Thieme F, Vogel M, Gausche R, et al. The Influence of Body Mass Index on the Growth Hormone Peak Response regarding Growth Hormone Stimulation Tests in Children. *Horm Res Paediatr*. 2022;95(5):452-460. doi:10.1159/000526240
39. Thirunagari R, Marrone A, Elsinghorst H, Mastrandrea LD. Hematuria as an adverse outcome following provocative growth hormone stimulation testing in children. *J Pediatr Endocrinol Metab*. 2018;31(5):539-543. doi:10.1515/jpem-2017-0458
40. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1049-1055. doi:10.1515/jpem.2011.327
41. Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M, Oron T. Diagnosis of Growth Hormone Deficiency in Children: The Efficacy of Glucagon versus Clonidine Stimulation Test. *Horm Res Paediatr*. 2020;93(7-8):470-476. doi:10.1159/000513393

42. Yau M, Chacko E, Regelmann MO, et al. Peak Growth Hormone Response to Combined Stimulation Test in 315 Children and Correlations with Metabolic Parameters. *Horm Res Paediatr*. 2019;92(1):36-44. doi:10.1159/000502308
43. Yau M, Rapaport R. Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. *Front Endocrinol (Lausanne)*. 2022;13:902364. Published 2022 Jun 9. doi:10.3389/fendo.2022.902364
44. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. *Endocr Connect*. 2023;12(7):e220504. Published 2023 Jun 12. doi:10.1530/EC-22-0504

GROWTH HORMONE STIMULATION TEST
Combined Protocol
Stimulants: Arginine and Clonidine

Indications:

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

Rationale:

The hypothalamus stimulates 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.

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, panhypopituitarism

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

People with known allergic tendencies

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

Caution in children with known congenital / acquired heart disease

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 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:

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

Formulation & 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)	Intravenous infusion over 30 minutes

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 available on PBS, Australia

Clonidine 25 microgram and 150 microgram tablets available in New Zealand

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Clonidine

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

Hypotension, postural hypotension. Fall in blood pressure by ~10 mmHg about 1 hour after ingestion. Usually resolves by the end of the test but may last several hours. Effect 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 patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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 on regular antihypertensive medication, please check with the SMO 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 request form.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	In the 2 days before the day of GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	In the 2-3 days before the day of GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, clonidine

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Ideally perform test first thing in the morning following an overnight fast. However, minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh patient, calculate arginine and glucagon doses and take baseline observations.
3. Insert IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer arginine via intravenous infusion over 30 minutes. The time that the infusion STARTS (not finishes) is Time 0. Allow time to give a 10 – 15 ml flush with 0.9% saline prior to taking the 30 minute blood sample.
5. Administer clonidine orally (dose as per dosing table above) as soon as+90Min blood sample has been collected.
6. Consider giving 10 ml/kg IV bolus of 0.9% sodium chloride over 30 minutes following 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.
7. Blood sampling as per table below.
8. 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.
9. 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.
10. Take care ambulating the child following completion of the test. Postural hypotension may occur.
11. No food until the test is completed. Water is permitted.

Discharge:

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 abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered				**Dose Administered**						**Time Administered**				
	Baseline		Minutes post START of arginine infusion											
Actual time bloods taken														
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	✓	Administer arginine	✓	✓	✓	✓	✓	Administer clonidine	✓	✓	✓	✓	✓
Cortisol	✓		✓		✓								
ACTH	✓												
Other tests, for example IGF-1, IGFBP-3 as per requesting clinician	+/-												
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL						

Interpretation:

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

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Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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 GH stimulation tests. In children who are 5 years and older, GH testing with sex steroid priming is required.	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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.

REFERENCES

1. AbdelNabi R, Al Khalifah R. Growth hormone stimulation test with Clonidine and Arginine an unreported side effect. *J. Clin. Transl. Endocrinol.: Case Rep*. 2020;15. https://doi.org/10.1016/j.jecr.2019.100055
2. Al Balwi R, Al-Qahtani M, Alrowished AK, et al. Reliability of Agreement between Insulin, Clonidine, and Glucagon Stimulation Tests for the Diagnosis of Growth Hormone Deficiency in Children: A Retrospective Cohort Study. *Children (Basel)*. 2023;10(8):1381. doi:10.3390/children10081381
3. Al Khalifah R, Moisan L, Bui H. The shortened combined clonidine and arginine test for growth hormone deficiency is practical and specific: a diagnostic accuracy study. *J Pediatr Endocrinol Metab*. 2016;29(3):305-310. doi:10.1515/jpem-2015-0284
4. Australia and New Zealand Society for Paediatric Endocrinology and Diabetes (ANZSPED) website: Clinical Resources & Links > Growth & Growth Charts. https://anzsped.org/clinical-resources-links/growth-growth-charts/
5. Barrett J, Maranda L, Nwosu BU. The Relationship between Subnormal Peak-Stimulated Growth Hormone Levels and Auxological Characteristics in Obese Children. *Front Endocrinol (Lausanne)*. 2014;5:35. Published 2014 Mar 25. doi:10.3389/fendo.2014.00035
6. Bhat N, Dulmovits E, Lane A, Messina C, Wilson T. Combined simultaneous arginine clonidine stimulation test: Timing of peak growth hormone (GH) concentration and correlation with clinical indices of GH status. *Growth Horm IGF Res*. 2018;40:28-31. doi:10.1016/j.ghir.2018.04.002
7. Binder G, Reinehr T, Ibáñez L, et al. GHD Diagnostics in Europe and the US: An Audit of National Guidelines and Practice. *Horm Res Paediatr*. 2019;92(3):150-156. doi:10.1159/000503783
8. Bizzarri C, Pedicelli S, Boscherini B, Bedogni G, Cappa M, Cianfarani S. Early retesting by GHRH + arginine test shows normal GH response in most children with idiopathic GH deficiency. *J Endocrinol Invest*. 2015;38(4):429-436. doi:10.1007/s40618-014-0205-3
9. Chesover AD, Dattani MT. Evaluation of growth hormone stimulation testing in children. *Clin Endocrinol (Oxf)*. 2016;84(5):708-714. doi:10.1111/cen.13035
10. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. *Best Pract Res Clin Endocrinol Metab*. 2016;30(6):737-747. doi:10.1016/j.beem.2016.11.002
11. Christoforidis A, Triantafyllou P, Slavakis A, Katzos G. Clonidine and glucagon stimulation for testing growth hormone secretion in children and adolescents: can we make it with fewer samples?. *J Endocrinol Invest*. 2013;36(11):1046-1050. doi:10.3275/9061
12. Dori EB, Avnon Ziv C, Auerbach A, Greenberg Y, Zaken H, Levy-Khademi F. The inter - Test variability of growth hormone stimulation tests and factors affecting this variability. *Growth Horm IGF Res*. 2020;55:101361. doi:10.1016/j.ghir.2020.101361
13. Duncan G, Kiff S, Mitchell RT. Sex steroid priming for growth hormone stimulation testing in children and adolescents with short stature: A systematic review. *Clin Endocrinol (Oxf)*. 2023;98(4):527-535. doi:10.1111/cen.14862

14. Frerichs C, Raymond L, Senniappan S. Variations in sex steroid priming for growth hormone stimulation testing in UK. *Arch Dis Child*. 2017;102(3):294. doi:10.1136/archdischild-2016-311186
15. Gabreanu GR. An update on the diagnosis of growth hormone deficiency. *Discoveries (Craiova)*. 2018;6(1):e82. Published 2018 Apr 12. doi:10.15190/d.2018.2
16. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;85(11):3990-3993. doi:10.1210/jcem.85.11.6984
17. Gillis D, Granat N, Strich D. The arginine stimulation test: timing of peak is not a helpful parameter in the diagnosis of growth hormone deficiency. *J Pediatr Endocrinol Metab*. 2013;26(9-10):813-817. doi:10.1515/jpem-2013-0075
18. Glibbery M, Fleming A, Chanchlani R, et al. Myalgia and Hematuria in Association with Clonidine and Arginine Administration for Growth Hormone Stimulation Tests. *Case Rep Med*. 2020;2020:4827072. Published 2020 May 26. doi:10.1155/2020/4827072
19. Kim JH, Chae HW, Chin SO, et al. Diagnosis and Treatment of Growth Hormone Deficiency: A Position Statement from Korean Endocrine Society and Korean Society of Pediatric Endocrinology. *Endocrinol Metab (Seoul)*. 2020;35(2):272-287. doi:10.3803/EnM.2020.35.2.272
20. Lee NY, Kim SE, Kim S, et al. Effect of body mass index on peak growth hormone level after growth hormone stimulation test in children with short stature. *Ann Pediatr Endocrinol Metab*. 2021;26(3):192-198. doi:10.6065/apem.2040246.123
21. Lee HS, Hwang JS. Influence of body mass index on growth hormone responses to classic provocative tests in children with short stature. *Neuroendocrinology*. 2011;93(4):259-264. doi:10.1159/000326838
22. Lennartsson O, Nilsson O, Lodefalk M. Discordance Between Stimulated and Spontaneous Growth Hormone Levels in Short Children Is Dependent on Cut-Off Level and Partly Explained by Refractoriness. *Front Endocrinol (Lausanne)*. 2020;11:584906. Published 2020 Nov 17. doi:10.3389/fendo.2020.584906
23. Loche S, Guzzetti C, Pilia S, et al. Effect of body mass index on the growth hormone response to clonidine stimulation testing in children with short stature. *Clin Endocrinol (Oxf)*. 2011;74(6):726-731. doi:10.1111/j.1365-2265.2011.03988.x
24. Marinkovic M, Newfield RS. Self-limiting hematuria following growth hormone provocative testing with arginine hydrochloride. *J Pediatr Endocrinol Metab*. 2012;25(7-8):791-793. doi:10.1515/jpem-2012-0160
25. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85(11):4168-4172. doi:10.1210/jcem.85.11.6928
26. Mauras N, Walton P, Nicar M, Welch S, Rogol AD. Growth hormone stimulation testing in both short and normal statured children: use of an immunofunctional assay. *Pediatr Res*. 2000;48(5):614-618. doi:10.1203/00006450-200011000-00010

27. Morris AH, Harrington MH, Churchill DL, Olshan JS. Growth hormone stimulation testing with oral clonidine: 90 minutes is the preferred duration for the assessment of growth hormone reserve. *J Pediatr Endocrinol Metab*. 2001;14(9):1657-1660. doi:10.1515/jpem.2001.14.9.1657
28. Muster L., Zangen D, Nesher R, et al. Arginine and Clonidine Stimulation Tests for Growth Hormone Deficiency Revisited - Do We Really Need So Many Samples?. *Journal of Pediatric Endocrinology and Metabolism*, 2009;*22*(3): 215-224. https://doi.org/10.1515/JPEM.2009.22.3.215
29. Nwosu BU, Coco M, Jones J, Barnes KM, Yanovski JA, Baron J. Short stature with normal growth hormone stimulation testing: lack of evidence for partial growth hormone deficiency or insensitivity. *Horm Res*. 2004;62(2):97-102. doi:10.1159/000079711
30. Pharmac Special Authority for Somatropin (SA 2032). https://schedule.pharmac.govt.nz/latest/SA2032.pdf
31. Rameez Raja B, Natarajan V, Sridhar S. Abstract 87: A comparative study to validate peak growth hormone levels in clonidine and glucagon stimulation test in severe short stature. *Indian J. Endocrinol. Metab.* 2022;26(Suppl 8):S36-S37. doi:10.4103/2230-8210.363778.
32. Richmond E, Rogol AD. Testing for growth hormone deficiency in children. *Growth Horm IGF Res*. 2020;50:57-60. doi:10.1016/j.ghir.2019.12.002
33. Rochiccioli P, Enjaume C, Tauber MT, Pienkowski C. Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. *Acta Paediatr*. 1993;82(3):245-248. doi:10.1111/j.1651-2227.1993.tb12652.x
34. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre- and early adolescent children is evidence based. *Horm Res Paediatr*. 2011;75(1):78-80. doi:10.1159/000323353
35. Snyder C, Hess C. Abstract: Tissue Necrosis: A Rare Adverse Event of Arginine Infusion during Growth Hormone Stimulation Testing. *J Pediatr Nurs.* 2016;31(3):364.
36. Sodero G, Mariani F, Caprarelli M, et al. Growth hormone responses during arginine and clonidine stimulation test: Correlations with patients' auxological and metabolic parameters in a single centre study. *Growth Horm IGF Res*. 2023;68:101522. doi:10.1016/j.ghir.2022.101522
37. Stanley T. Diagnosis of growth hormone deficiency in childhood. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(1):47-52. doi:10.1097/MED.0b013e32834ec952
38. Thakur DS, Bhagwat NM, Bhide MM, et al. Clonidine Stimulation Test: Is Single Best Time Point, Convenient Yet Efficacious?. *Indian J Endocrinol Metab*. 2018;22(4):511-514. doi:10.4103/ijem.IJEM_101_18
39. Thieme F, Vogel M, Gausche R, et al. The Influence of Body Mass Index on the Growth Hormone Peak Response regarding Growth Hormone Stimulation Tests in Children. *Horm Res Paediatr*. 2022;95(5):452-460. doi:10.1159/000526240
40. Thirunagari R, Marrone A, Elsinghorst H, Mastrandrea LD. Hematuria as an adverse outcome following provocative growth hormone stimulation testing in children. *J Pediatr Endocrinol Metab*. 2018;31(5):539-543. doi:10.1515/jpem-2017-0458
41. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. *J Pediatr Endocrinol Metab*. 2012;25(11-12):1049-1055. doi:10.1515/jpem.2011.327

42. Yackobovitch-Gavan M, Lazar L, Diamant R, Phillip M, Oron T. Diagnosis of Growth Hormone Deficiency in Children: The Efficacy of Glucagon versus Clonidine Stimulation Test. *Horm Res Paediatr*. 2020;93(7-8):470-476. doi:10.1159/000513393
43. Yau M, Chacko E, Regelmann MO, et al. Peak Growth Hormone Response to Combined Stimulation Test in 315 Children and Correlations with Metabolic Parameters. *Horm Res Paediatr*. 2019;92(1):36-44. doi:10.1159/000502308
44. Yau M, Rapaport R. Growth Hormone Stimulation Testing: To Test or Not to Test? That Is One of the Questions. *Front Endocrinol (Lausanne)*. 2022;13:902364. Published 2022 Jun 9. doi:10.3389/fendo.2022.902364
45. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. *Endocr Connect*. 2023;12(7):e220504. Published 2023 Jun 12. doi:10.1530/EC-22-0504

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:

Growth hormone releasing hormone (GHRH) from the hypothalamus stimulates production of GH by somatotrophs in the anterior pituitary and GH subsequently stimulates the synthesis of IGF-1 which is primarily produced 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.

Contraindications:

Consider terminating test if fasting hyperglycaemia >10 mmol/L on glucose meter.

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.

Recent surgery or trauma which may impair glucose tolerance.

Note: beta-blockers, corticosteroids, phenytoin, thiazides, oestrogens and intercurrent illness can impair glucose tolerance. Caution should be taken.

Expertise level:

Minimal requirement for test to 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 / monohydrate forms of glucose in the OGTT has 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 patient's 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 with an OGTT.

Normal physical activity, no intercurrent illness.

The test should be performed in the morning after a 10-16 hour overnight fast. Water is permitted.

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

Equipment:

Equipment for IV cannulation and blood sampling

- IV cannula, blood tubes, 2ml and 5ml syringes, 0.9% saline for IV cannula flushes etc

Access to hypoglycaemia treatment supplies (see Notes section below)

Observations:

On arrival: BP, pulse, weight, height

Blood glucose level via glucometer on each blood sample

Method:

1. Weigh patient and take baseline observations.
2. Calculate and measure out volume of glucose solution to be consumed (if not already pre-prepared).
3. Insert IV cannula.
4. Collect baseline (pre-stimulation) bloods and also measure glucose level on bedside/point of care glucometer.
5. Glucose drink to be consumed over **no more** than 10 minutes.
6. Emphasize patient is to be resting during the test. Water is permitted.
7. Blood samples collected at timed intervals as per table below. Glucose level to be measured on bedside/point of care glucometer at each sampling time point as well. Blood samples are timed from the moment of the first swallow, which is defined as time 0 minutes.
8. Patient to be fed before discharge. Remove IV cannula if diet and fluids are tolerated.

Discharge:

Child must have eaten and have a normal blood glucose level. All observations should be within normal limits, if abnormal repeat as required. Review by medical personnel or fulfilment of criteria-led discharge parameters prior to discharge.

Sample collection:

	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	✓		✓	✓	✓	✓	✓	✓
Growth hormone	✓		✓	✓	✓	✓	✓	✓
IGF1	✓							
Other tests, for example HbA1c, c-peptide as per SMO responsible for patient	+/-		+/-	+/-	+/-	+/-	+/-	+/-
Sample tubes / Minimum blood volumes								

Note:

Minimum paediatric data is available for the use of the 150 min and 180 min blood samples; if time constraints or difficulties obtaining blood samples, the 150 min and 180 min post glucose load blood samples can be omitted

Interpretation:

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

Specific male and female GH cut-offs based on Tanner stage gave been proposed (Misra M et al, JCEM 2007):

	Mean GH level at nadir (mcg/L)	Upper limit for GH nadir (mcg/L)	Minutes post glucose load to reach nadir
Female			
Tanner 1	0.09	0.64	60
Tanner 2 - 3	0.22	1.57	60
Tanner 4 - 5	0.16	0.64	30
Male			
Tanner 1 - 2	0.10	0.50	90
Tanner 3 - 4	0.21	0.50	90
Tanner 5	0.10	0.50	90

Note:

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

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.

REFERENCES

1. Akirov A, Masri-Iraqi H, Dotan I, Shimon I. The Biochemical Diagnosis of Acromegaly. *J Clin Med*. 2021;10(5):1147. Published 2021 Mar 9. doi:10.3390/jcm10051147
2. Holl R, Bucher P, Wolfgang S, et al. Suppression of growth hormone by oral glucose in the evaluation of tall stature. Hormone Research 1999; 51(1):20-24
3. Misra M, Cord J, Prabhakaran R, et al. Growth Hormone Suppression after an Oral Glucose Load in Children. JCEM 2007; 92(12):4623-4629. Doi: 1-.1210/jc.2007-1244
4. Misra M, Miller K, Herzog D, et al. Growth hormone and ghrelin responses to an oral glucose load in adolescent girls with anorexia and controls. J Clin Endocrinol Metab 2004; 89:1605-1612

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.

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 activation and function of the HPG axis.

Contraindications:

Pregnancy (relative contraindication)

Expertise level:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and IV cannulation skills.

Formulation & Dose:

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)	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 subjects 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 request form.

Equipment:

Equipment for IV cannulation + blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – triptorelin OR gonadorelin

Observations:

Temperature, BP, HR at baseline and then hourly throughout the test

Method:

1. Weigh patient and take baseline observations.
2. Insert IV cannula and take baseline (pre-stimulation) bloods samples.
3. Administer GnRH agonist (dose/route as per table above)
4. Blood sampling as below. If performed as part of a combined pituitary test, see combined protocol
5. Remove 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 GnRH agonist. See ‘Timing of post-GnRH agonist stimulation blood sampling note’ below for further details.

Discharge:

Once the test is complete, ensure the patient meets 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:

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

*See Notes section below (Timing of post-GnRH agonist stimulation blood samples)

Interpretation:

LH peak post-GnRH agonist ≥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.

See Notes section below regarding the use and interpretation of GnRH stimulation test for 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 (may also occur in 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 stage 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 estradiol 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 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/GnRHa used, the sampling timepoints used, and the LH assay used.

If only taking blood samples at baseline and 1-2 timepoint post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, from 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 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 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 isn't required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRHa stimulation testosterone/estradiol 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 when it comes to 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 intravenous injection (0.1mg/m^2 body surface area, maximum dose 0.1mg):

	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)

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

REFERENCES

1. Ab Rahim SN, Omar J, Tuan Ismail TS. Gonadotropin-releasing hormone stimulation test and diagnostic cutoff in precocious puberty: a mini review. *Ann Pediatr Endocrinol Metab*. 2020;25(3):152-155. doi:10.6065/apem.2040004.002
2. Becker M, Hesse V. Minipuberty: Why Does it Happen?. *Horm Res Paediatr*. 2020;93(2):76-84. doi:10.1159/000508329
3. Bizzarri C, Spadoni GL, Bottaro G, et al. The response to gonadotropin releasing hormone (GnRH) stimulation test does not predict the progression to true precocious puberty in girls with onset of premature thelarche in the first three years of life. *J Clin Endocrinol Metab*. 2014;99(2):433-439. doi:10.1210/jc.2013-3292
4. Brito VN, Spinola-Castro AM, Kochi C, Kopacek C, Silva PC, Guerra-Júnior G. Central precocious puberty: revisiting the diagnosis and therapeutic management [published correction appears in Arch Endocrinol Metab. 2016 Aug;60(4):407]. *Arch Endocrinol Metab*. 2016;60(2):163-172. doi:10.1590/2359-3997000000144
5. Cantas-Orsdemir S, Eugster EA. Update on central precocious puberty: from etiologies to outcomes. *Expert Rev Endocrinol Metab*. 2019;14(2):123-130. doi:10.1080/17446651.2019.1575726
6. Cao R, Liu J, Fu P, Zhou Y, Li Z, Liu P. The Diagnostic Utility of the Basal Luteinizing Hormone Level and Single 60-Minute Post GnRH Agonist Stimulation Test for Idiopathic Central Precocious Puberty in Girls. *Front Endocrinol (Lausanne)*. 2021;12:713880. Published 2021 Aug 12. doi:10.3389/fendo.2021.713880
7. Carretto F, Salinas-Vert I, Granada-Yvern ML, et al. The usefulness of the leuprolide stimulation test as a diagnostic method of idiopathic central precocious puberty in girls. *Horm Metab Res*. 2014;46(13):959-963. doi:10.1055/s-0034-1387790
8. Choi JH, Shin YL, Yoo HW. Predictive factors for organic central precocious puberty and utility of simplified gonadotropin-releasing hormone tests. *Pediatr Int*. 2007;49(6):806-810. doi:10.1111/j.1442-200X.2007.02475.x
9. Ding Y, Li J, Yu Y, et al. Evaluation of basal sex hormone levels for activation of the hypothalamic-pituitary-gonadal axis. *J Pediatr Endocrinol Metab*. 2018;31(3):323-329. doi:10.1515/jpem-2017-0124
10. Freire AV, Escobar ME, Gryngarten MG, et al. High diagnostic accuracy of subcutaneous Triptorelin test compared with GnRH test for diagnosing central precocious puberty in girls. *Clin Endocrinol (Oxf)*. 2013;78(3):398-404. doi:10.1111/j.1365-2265.2012.04517.x
11. Harrington J, Palmert MR, Hamilton J. Use of local data to enhance uptake of published recommendations: an example from the diagnostic evaluation of precocious puberty. *Arch Dis Child*. 2014;99(1):15-20. doi:10.1136/archdischild-2013-304414
12. Huynh QTV, Le NQK, Huang SY, et al. Development and Validation of Clinical Diagnostic Model for Girls with Central Precocious Puberty: Machine-learning Approaches. *PLoS One*. 2022;17(1):e0261965. Published 2022 Jan 21. doi:10.1371/journal.pone.0261965
13. Iughetti L, Predieri B, Ferrari M, et al. Diagnosis of central precocious puberty: endocrine assessment. *J Pediatr Endocrinol Metab*. 2000;13 Suppl 1:709-715. doi:10.1515/jpem.2000.13.s1.709

14. Kandemir N, Demirbilek H, Özön ZA, Gönç N, Alikaşifoğlu A. GnRH stimulation test in precocious puberty: single sample is adequate for diagnosis and dose adjustment. *J Clin Res Pediatr Endocrinol*. 2011;3(1):12-17. doi:10.4274/jcrpe.v3i1.03
15. Kim HK, Kee SJ, Seo JY, Yang EM, Chae HJ, Kim CJ. Gonadotropin-releasing hormone stimulation test for precocious puberty. *Korean J Lab Med*. 2011;31(4):244-249. doi:10.3343/kjlm.2011.31.4.244
16. Kim MS, Hwang PH, Lee DY. A Gonadotropin-Releasing Hormone (GnRH) Stimulation Test Before and After GnRH Analogue Treatment for Central Precocious Puberty: Has the GnRH Test been Adequately Simplified?. *Indian J Pediatr*. 2015;82(11):996-1000. doi:10.1007/s12098-015-1761-z
17. Latronico AC, Brito VN, Carel JC. Causes, diagnosis, and treatment of central precocious puberty. *Lancet Diabetes Endocrinol*. 2016;4(3):265-274. doi:10.1016/S2213-8587(15)00380-0
18. Lee DM, Chung IH. Morning basal luteinizing hormone, a good screening tool for diagnosing central precocious puberty. *Ann Pediatr Endocrinol Metab*. 2019;24(1):27-33. doi:10.6065/apem.2019.24.1.27
19. Lee HS, Yoon JS, Hwang JS. Luteinizing Hormone Secretion during Gonadotropin-Releasing Hormone Stimulation Tests in Obese Girls with Central Precocious Puberty. *J Clin Res Pediatr Endocrinol*. 2016;8(4):392-398. doi:10.4274/jcrpe.3091
20. Menon PS. Precocious Puberty, GnRH Stimulation Test and Monitoring GnRH Analog Therapy. *Indian J Pediatr*. 2015;82(11):980-982. doi:10.1007/s12098-015-1903-3
21. Pasternak Y, Friger M, Loewenthal N, Haim A, Hershkovitz E. The utility of basal serum LH in prediction of central precocious puberty in girls. *Eur J Endocrinol*. 2012;166(2):295-299. doi:10.1530/EJE-11-0720
22. Radicioni A, Lenzi A, Spaziani M, et al. A multicenter evaluation of immunoassays for follicle-stimulating hormone, luteinizing hormone and testosterone: concordance, imprecision and reference values. *J Endocrinol Invest*. 2013;36(9):739-744. doi:10.1007/BF03347112
23. Vestergaard ET, Schjørring ME, Kamperis K, et al. The follicle-stimulating hormone (FSH) and luteinizing hormone (LH) response to a gonadotropin-releasing hormone analogue test in healthy prepubertal girls aged 10 months to 6 years. *Eur J Endocrinol*. 2017;176(6):747-753. doi:10.1530/EJE-17-0042
24. Vukovic R, Milenkovic T, Soldatovic I, Pekic S, Mitrovic K, Todorovic S. Triptorelin stimulated luteinizing hormone concentrations for diagnosing central precocious puberty: study of diagnostic accuracy. *Endocrine*. 2022;75(3):934-941. doi:10.1007/s12020-021-02947-z
25. Yeh SN, Ting WH, Huang CY, et al. Diagnostic evaluation of central precocious puberty in girls. *Pediatr Neonatol*. 2021;62(2):187-194. doi:10.1016/j.pedneo.2020.12.001
26. Zhao C, Tang Y, Cheng L. Diagnostic Value of LH Peak Value of the GnRH Stimulation Test for Girls with Precocious Puberty and Its Correlation with Body Mass Index. *Comput Math Methods Med.* 2022;2022:4118911. Published 2022 Jun 2. doi:10.1155/2022/4118911

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, 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 and constitutional delay of growth and puberty

Rationale:

Luteinising hormone (LH) is a gonadotropin from the anterior pituitary which stimulates Leydig cells in testicular tissue to secrete testosterone. Human chorionic gonadotropin (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

Formulation:

Recombinant human chorionic gonadotrophin (r-hCG)

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:

Single dose protocol

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

Note: Historically, 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)

Adverse reactions:

Local reaction at injection site (irritation, pain, erythema), GI upset, headache

Other side effects related 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 request form.

If a GnRH stimulation test is also planned

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

- Collect baseline blood samples for BOTH TESTS prior to GnRH or hCG being given
- 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 for blood collection e.g. butterfly and syringe / IV cannula, blood tubes

The stimulant - Ovidrel pre-filled pen

Observations:

No specific observations are required.

Method:

1. Collect baseline (pre-hCG) bloods
2. Administer hCG as per 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 discharge criteria as per your local unit. Make sure arrangements have been made for the post-hCG blood test to be done.

Sample collection:

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 as per consultant responsible for patient	+/-		+/-
Sample Tubes / Minimum Blood Volume			

Interpretation:

Table 1: Testosterone and DHT/T cut-off values when stimulant used is rhCG

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 > 30*	Suggestive of 5α-reductase-2 deficiency; warrants genetic test
*Ratio refers to both mass units and SI units as the conversion factor for both testosterone and dihydrotestosterone are the same.				

Paediatric study published using LC-MS/MS to measure the gonadal response to hCG stimulation:

Oliveira et al. Androgens by immunoassay and mass spectrometry in children with 46,XY DSD. Endocrine Connections. 2020; 9 (11): 1085-1094.

- 19 pre-pubertal 46, XY patients, rhCG 250mcg (age/weight of patients not mentioned), baseline + 7-days post-rhCG bloods for testosterone, DHT, androstenedione, DHEA – all samples tested using IA and LC-MS/MS (all had prior proven normal T secretion evaluated by conventional IA after uhCG stim test in childhood or hormonal assessment done during mini-puberty, T > 5.2 nmol/L)

- Results: IA and LC-MS/MS can't be considered equivalent, IA affected by proportional (androstenedione) and systematic (testosterone, androstenedione) concordance errors, tending to overestimate testosterone + androstenedione values and underestimate DHEA and DHT values compared to LC-MS/MS

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

GnRH agonist stimulation test is more commonly used to assess for hypogonadotropic hypogonadism

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

While hCG stimulates ovarian oestrogen 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.

REFERENCES

1. Bertelloni S, Russo G, Baroncelli GI. Human Chorionic Gonadotropin Test: Old Uncertainties, New Perspectives, and Value in 46,XY Disorders of Sex Development. *Sex Dev*. 2018;12(1-3):41-49. doi:10.1159/000481552
2. Cailleux-Bounacer A, Reznik Y, Cauliez B, Menard JF, Duparc C, Kuhn JM. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. *Eur J Endocrinol*. 2008;159(2):171-178. doi:10.1530/EJE-07-0876
3. de Oliveira LR, Longui CA, Guaragna-Filho G, et al. Suggested Cutoff Point for Testosterone by Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) after Stimulation with Recombinant Human Chorionic Gonadotropin. *Sex Dev*. 2022;16(4):266-269. doi:10.1159/000519422
4. Goyal A, Kubihal S, Gupta Y, Jyotsna VP, Khadgawat R. Dynamic Testing for Evaluation of Adrenal and Gonadal Function in Pediatric and Adult Endocrinology: An Overview. *Indian J Endocrinol Metab*. 2019;23(6):593-601. doi:10.4103/ijem.IJEM_553_19
5. Hannema SE, de Rijke YB. Improving Laboratory Assessment in Disorders of Sex Development through a Multidisciplinary Network. *Sex Dev*. 2018;12(1-3):135-139. doi:10.1159/000486329
6. Ishii T, Matsuo N, Sato S, et al. Human Chorionic Gonadotropin Stimulation Test in Prepubertal Children with Micropenis Can Accurately Predict Leydig Cell Function in Pubertal or Postpubertal Adolescents. *Horm Res Paediatr*. 2015;84(5):305-310. doi:10.1159/000439234
7. Kulle AE, Riepe FG, Melchior D, Hiort O, Holterhus PM. A novel ultrapressure liquid chromatography tandem mass spectrometry method for the simultaneous determination of androstenedione, testosterone, and dihydrotestosterone in pediatric blood samples: age- and sex-specific reference data. *J Clin Endocrinol Metab*.
8. Maimoun L, Philibert P, Cammas B, et al. Phenotypical, biological, and molecular heterogeneity of 5α-reductase deficiency: an extensive international experience of 55 patients. *J Clin Endocrinol Metab*. 2011;96(2):296-307. doi:10.1210/jc.2010-1024
9. Oliveira LR, Homma TK, Woloszynek RR, Brito VN, Longui CA. Gonadal response after a single-dose stimulation test with recombinant human chorionic gonadotropin (rhCG) in patients with isolated prepubertal cryptorchidism. *Basic Clin Androl*. 2016;26:13. Published 2016 Oct 28. doi:10.1186/s12610-016-0039-2
10. Oliveira LR, Longui CA, Guaragna-Filho G, et al. Androgens by immunoassay and mass spectrometry in children with 46,XY disorder of sex development. *Endocr Connect*. 2020;9(11):1085-1094. doi:10.1530/EC-20-0454
11. Ranke MB, Mullis P-E (eds): Diagnostics of Endocrine Function in Children and Adolescents, ed 4. Basel, Karger, 2011, pp310-330.
12. Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab*. 2009;94(3):780-785. doi:10.1210/jc.2008-0302

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 secondary adrenal insufficiency (ACTH deficiency).

Rationale:

ACTH is the primary regulator of glucocorticoid production, and also plays a role in adrenal androgen production. Tetracosactrin (Synacthen), 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 CRH/ACTH deficiency 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 Tetracosactrin (Synacthen) which can be falsely reassuring.

Contraindications:

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

Expertise level:

Anaphylaxis to Tetracosactrin 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:

Tetracosactrin (Synacthen, solution for injection) 250 mcg in 1 mL.

A synthetic polypeptide consisting of the first 24 amino acids of the ACTH molecule.

Dose:

Standard dose Synacthen test

Age	Dose	Route
0 – 6 months	62.5 micrograms	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 (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol immunoassay. This is not an issue with LC-MS/MS method).

This test should be performed before 0900am 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 SMO responsible for the patient if any additional tests are required **before** commencing the test. Specify which tests, if any, are required on request form.

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2 ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – Tetracosactrin (Synacthen)

Observations:

Baseline BP, HR, RR and hourly thereafter during the test

Method:

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

Sample collection:

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 1.5mL	SST 1.5mL

Interpretation:

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in 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 and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than the 30 minute levels.

	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
This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA)						

Caution in the interpretation of 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.

Historically, some SST protocols have stipulated that for an adrenal response to be deemed adequate / sufficient, in addition to having a peak cortisol level rise above a certain cut-off threshold, a minimum increment in cortisol level from baseline to peak had to also be achieved. This is however no longer a requirement as individuals with normal adrenal function with a high baseline cortisol level will not achieve this increment.

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 intravenous access is not obtainable, administer Synacthen intramuscularly and collect pre / post-Synacthen blood samples via finger-prick, heel prick, or venipuncture.

Neonates

In neonates <6 months, initial sub-optimal cortisol response (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.

Timing of SST post-neurosurgery

In patients who have recently undergone neurosurgery and are at risk of ACTH deficiency, check with the SMO responsible for the patient about the desired timeframe post-surgery that the SST should be arranged for. Following 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 approximately the first 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 a 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, other stressors.

REFERENCES

1. Alesci S, Ilias I, Souvatzoglou E, et al. Intramuscular administration of ACTH1-24 vs. 24-hour blood sampling in the assessment of adrenocortical function. *Hormones (Athens)*. 2005;4(2):96-100.
2. Birtolo MF, Antonini S, Saladino A, et al. ACTH Stimulation Test for the Diagnosis of Secondary Adrenal Insufficiency: Light and Shadow. *Biomedicines*. 2023;11(3):904. Published 2023 Mar 15. doi:10.3390/biomedicines11030904
3. Bornstein SR, Allolio B, Arlt W, et al. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016;101(2):364-389. doi:10.1210/jc.2015-1710
4. Bowden SA, Henry R. Pediatric Adrenal Insufficiency: Diagnosis, Management, and New Therapies. *Int J Pediatr*. 2018;2018:1739831. Published 2018 Nov 1. doi:10.1155/2018/1739831
5. Brown S, Hadlow N, Badshah I, Henley D. A time-adjusted cortisol cut-off can reduce referral rate for Synacthen stimulation test whilst maintaining diagnostic performance. *Clin Endocrinol (Oxf)*. 2017;87(5):418-424. doi:10.1111/cen.13405
6. Butt MI, Alzuhayri N, Amer L, et al. Comparing the utility of 30- and 60-minute cortisol levels after the standard short synacthen test to determine adrenal insufficiency: A retrospective cross-sectional study. *Medicine (Baltimore)*. 2020;99(43):e22621. doi:10.1097/MD.0000000000022621
7. Chanson P, Guignat L, Goichot B, et al. Group 2: Adrenal insufficiency: screening methods and confirmation of diagnosis. *Ann Endocrinol (Paris)*. 2017;78(6):495-511. doi:10.1016/j.ando.2017.10.005
8. Chiang C, Inder W, Grossmann M, Clifton-Bligh R, Coates P, Lim EM, Ward P, Stanford P, Florkowski C, Doery J. Harmonisation of Endocrine Dynamic Testing - Adult (HEDTA). The Endocrine Society of Australia and The Australasian Association of Clinical Biochemists, Australia, 2021
9. Chitale A, Musonda P, McGregor AM, Dhatariya KK. Determining the utility of the 60 min cortisol measurement in the short synacthen test. *Clin Endocrinol (Oxf)*. 2013;79(1):14-19. doi:10.1111/j.1365-2265.2012.04478.x
10. Courtney CH, McAllister AS, Bell PM, et al. Low- and standard-dose corticotropin and insulin hypoglycemia testing in the assessment of hypothalamic-pituitary-adrenal function after pituitary surgery. *J Clin Endocrinol Metab*. 2004;89(4):1712-1717. doi:10.1210/jc.2003-031577
11. Cross AS, Helen Kemp E, White A, et al. International survey on high- and low-dose synacthen test and assessment of accuracy in preparing low-dose synacthen. *Clin Endocrinol (Oxf)*. 2018;88(5):744-751. doi:10.1111/cen.13559
12. El-Farhan N, Pickett A, Ducroq D, et al. Method-specific serum cortisol responses to the adrenocorticotrophin test: comparison of gas chromatography-mass spectrometry and five automated immunoassays. *Clin Endocrinol (Oxf)*. 2013;78(5):673-680. doi:10.1111/cen.12039
13. Grunwald K, Rabe T, Urbancsek J, Runnebaum B, Vecsei P. Normal values for a short-time ACTH intravenous and intramuscular stimulation test in women in the reproductive age. *Gynecol Endocrinol*. 1990;4(4):287-306. doi:10.3109/09513599009024983

14. Hawley JM, Owen LJ, Lockhart SJ, et al. Serum Cortisol: An Up-To-Date Assessment of Routine Assay Performance. *Clin Chem*. 2016;62(9):1220-1229. doi:10.1373/clinchem.2016.255034
15. Lindholm J. Problems in Interpretation of the short ACTH test: an update and historical notes. *Exp Clin Endocrinol Diabetes*. 2015;123(8):441-445. doi:10.1055/s-0035-1548817
16. Michaelidou M, Yadegarfar G, Morris L, et al. Recalibration of thinking about adrenocortical function assessment: how the 'random' cortisol relates to the short synacthen test results. *Cardiovasc Endocrinol Metab*. 2021;10(2):137-145. Published 2021 Apr 12. doi:10.1097/XCE.0000000000000250
17. Neary N, Nieman L. Adrenal insufficiency: etiology, diagnosis and treatment. *Curr Opin Endocrinol Diabetes Obes*. 2010;17(3):217-223. doi:10.1097/MED.0b013e328338f608
18. Ng SM, Agwu JC, Dwan K. A systematic review and meta-analysis of Synacthen tests for assessing hypothalamic-pituitary-adrenal insufficiency in children. *Arch Dis Child*. 2016;101(9):847-853. doi:10.1136/archdischild-2015-308925
19. Ospina NS, Al Nofal A, Bancos I, et al. ACTH Stimulation Tests for the Diagnosis of Adrenal Insufficiency: Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2016;101(2):427-434. doi:10.1210/jc.2015-1700
20. Özsu E, Şıklar Z, Bilici E, et al. Intramuscular Short-term ACTH Test for the Determination of Adrenal Function in Children: Safe, Effective and Reliable. *J Clin Res Pediatr Endocrinol*. 2020;12(3):241-247. doi:10.4274/jcrpe.galenos.2019.2019.0099
21. Ramadoss V, Lazarus K, Prevost AT, Tan T, Meeran K, Choudhury S. Improving the Interpretation of Afternoon Cortisol Levels and SSTs to Prevent Misdiagnosis of Adrenal Insufficiency. *J Endocr Soc*. 2021;5(11):bvab147. Published 2021 Sep 4. doi:10.1210/jendso/bvab147
22. Sharma R, Madathil S, Maheshwari V, Roy K, Kumar B, Jain V. Long-acting intramuscular ACTH stimulation test for the diagnosis of secondary adrenal insufficiency in children. *J Pediatr Endocrinol Metab*. 2019;32(1):57-63. doi:10.1515/jpem-2018-0330
23. Stoupa A, González-Briceño L, Pinto G, et al. Inadequate cortisol response to the tetracosactide (Synacthen®) test in non-classic congenital adrenal hyperplasia: an exception to the rule?. *Horm Res Paediatr*. 2015;83(4):262-267. doi:10.1159/000369901
24. Tan TSE, Manfredonia C, Kumar R, et al. Retrospective review of Synacthen testing in infants. *Arch Dis Child*. 2018;103(10):984-986. doi:10.1136/archdischild-2017-313819
25. Ueland GÅ, Methlie P, Øksnes M, et al. The Short Cosyntropin Test Revisited: New Normal Reference Range Using LC-MS/MS. *J Clin Endocrinol Metab*. 2018;103(4):1696-1703. doi:10.1210/jc.2017-02602
26. Wang TWM, Wong MS, Smith JF, Howlett TA. The use of the short tetracosactrin test for the investigation of suspected pituitary hypofunction. *Ann Clin Biochem*. 1996;33 (Pt 2):112-118. doi:10.1177/000456329603300203

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. Tetracosactrin (Synacthen), a synthetic form of ACTH, is used to evaluate 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 the 17-OHP, the precursor steroid proximal to the defective enzyme.

Contraindications:

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

Expertise level:

Anaphylaxis to Tetracosactrin 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:

Tetracosactrin (Synacthen) 250 mcg in 1 mL.

A synthetic polypeptide consisting of the first 24 amino acids of the ACTH molecule.

Dose:

Standard dose Synacthen test

Age	Dose	Route
0 – 6 months	62.5 micrograms	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 hours in the case of dexamethasone) prior to testing (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

This test should be performed before 0900am 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 able to be measured.

Fasting is not required.

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

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – tetracosactrin (Synacthen)

Observations:

Baseline BP, HR, RR and hourly thereafter during the test

Method:

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

Sample collection:

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 1.5mL	SST 1.5mL

Interpretation:

17-hydroxyprogesterone levels

*Unstimulated 17-hydroxyprogesterone levels: s*uggested 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-hydroxyprogesterone levels: 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

21-deoxycortisol 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-hydroxyprogesterone. It has recently been investigated in the SST for identifying carriers of CYP21A2 mutations (HZ) and those with non-classical forms (NC).

21-deoxycortisol and 17-hydroxycortisol cutoffs by LC-MS/MS (1.73 nmol/L and 9.38 nmol/L, respectively) correctly recognised 82.5% HZ plus NC, but 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 (ng/dL) to SI units (nmol/L) to mass units (ng/dL) are 21-deoxcortisol (divide by 0.0289), 17-hydroxyprogesterone (divide by 0.030), and SI units (nmol/L) to mass unit (µg/L) for cortisol (divide by 27.6). Note cortisol is expressed as µg/L ($x10^3$) = ng/dL.

Cortisol level

The use of the historical peak cortisol cut-off threshold of 550 nmol/L in newer cortisol-specific assays may result in 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 and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than the 30 minute levels.

	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
This table has been adapted from the Harmonisation of Dynamic Endocrine Tests in Adults (HEDTA)						

Cortisol

Caution in the interpretation of 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.

Historically, some SST protocols have stipulated that for an adrenal response to be deemed adequate (i.e. consistent with adrenal sufficiency), in addition to having a peak cortisol level rise above a certain cut-off

threshold, there had to also be a minimum increment in cortisol level from baseline to peak. This requirement is however redundant as normal individuals with a high baseline cortisol level will not achieve this increment.

17-hydroxyprogesterone

17-hydroxyprogesterone 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 17OHP 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.

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 / post-Synacthen blood samples via finger-prick, heel prick, or venepuncture.

Neonates note

In neonates <6 months, initial sub-optimal cortisol response (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.

REFERENCES

1. Bachega TA, Billerbeck AE, Marcondes JA, Madureira G, Arnhold IJ, Mendonca BB. Influence of different genotypes on 17-hydroxyprogesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2000;52(5):601-607. doi:10.1046/j.1365-2265.2000.00995.x
2. Bachega TA, Brenlha EM, Billerbeck AE, et al. Variable ACTH-stimulated 17-hydroxyprogesterone values in 21-hydroxylase deficiency carriers are not related to the different CYP21 gene mutations. *J Clin Endocrinol Metab*. 2002;87(2):786-790. doi:10.1210/jcem.87.2.8247
3. Castro PS, Rassi TO, Araujo RF, et al. High frequency of non-classical congenital adrenal hyperplasia form among children with persistently elevated levels of 17-hydroxyprogesterone after newborn screening. *J Pediatr Endocrinol Metab*. 2019;32(5):499-504. doi:10.1515/jpem-2018-0398
4. Chesover AD, Millar H, Sepiashvili L, Adeli K, Palmert MR, Hamilton J. Screening for Nonclassic Congenital Adrenal Hyperplasia in the Era of Liquid Chromatography-Tandem Mass Spectrometry. *J Endocr Soc*. 2019;4(2):bvz030. Published 2019 Dec 18. doi:10.1210/jendso/bvz030
5. Chiang C, Inder W, Grossmann M, Clifton-Bligh R, Coates P, Lim EM, Ward P, Stanford P, Florkowski C, Doery J. Harmonisation of Endocrine Dynamic Testing - Adult (HEDTA). The Endocrine Society of Australia and The Australasian Association of Clinical Biochemists, Australia, 2021
6. Costa-Barbosa FA, Carvalho VM, Oliveira KC, Vieira JGH, Kater CE. Reassessment of predictive values of ACTH-stimulated serum 21-deoxycortisol and 17-hydroxyprogesterone to identify CYP21A2 heterozygote carriers and nonclassic subjects. *Clin Endocrinol (Oxf)*. 2021;95(4):677-685. doi:10.1111/cen.14550
7. Dörr HG, Schulze N, Bettendorf M, et al. Genotype-phenotype correlations in children and adolescents with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Mol Cell Pediatr*. 2020;7(1):8. Published 2020 Jul 9. doi:10.1186/s40348-020-00100-w
8. Eisenhofer G, Peitzsch M, Kaden D, et al. Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: Impact of gender, age, oral contraceptives, body mass index and blood pressure status. *Clin Chim Acta*. 2017;470:115-124. doi:10.1016/j.cca.2017.05.002
9. El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia [published correction appears in Lancet. 2017 Nov 11;390(10108):2142]. *Lancet*. 2017;390(10108):2194-2210. doi:10.1016/S0140-6736(17)31431-9
10. Etter ML, Eichhorst J, Lehotay DC. Clinical determination of 17-hydroxyprogesterone in serum by LC-MS/MS: comparison to Coat-A-Count RIA method. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2006;840(1):69-74. doi:10.1016/j.jchromb.2006.04.038
11. Fiet J, Le Bouc Y, Guéchot J, et al. A Liquid Chromatography/Tandem Mass Spectometry Profile of 16 Serum Steroids, Including 21-Deoxycortisol and 21-Deoxycorticosterone, for Management of Congenital Adrenal Hyperplasia. *J Endocr Soc*. 2017;1(3):186-201. Published 2017 Feb 10. doi:10.1210/js.2016-1048
12. Grandone A, Marzuillo P, Luongo C, et al. Basal levels of 17-hydroxyprogesterone can distinguish children with isolated precocious pubarche. *Pediatr Res*. 2018;84(4):533-536. doi:10.1038/s41390-018-0096-7

13. Kulle AE, Riepe FG, Hedderich J, et al. LC-MS/MS based determination of basal- and ACTH-stimulated plasma concentrations of 11 steroid hormones: implications for detecting heterozygote CYP21A2 mutation carriers. *Eur J Endocrinol*. 2015;173(4):517-524. doi:10.1530/EJE-14-1084
14. Kyriakopoulou L, Yazdanpanah M, Colantonio DA, Chan MK, Daly CH, Adeli K. A sensitive and rapid mass spectrometric method for the simultaneous measurement of eight steroid hormones and CALIPER pediatric reference intervals. *Clin Biochem*. 2013;46(7-8):642-651. doi:10.1016/j.clinbiochem.2013.01.002
15. Nordenström A, Falhammar H. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2019;180(3):R127-R145. doi:10.1530/EJE-18-0712
16. Speiser PW, Arlt W, Auchus RJ, et al. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline [published correction appears in J Clin Endocrinol Metab. 2019 Jan 1;104(1):39-40]. *J Clin Endocrinol Metab*. 2018;103(11):4043-4088. doi:10.1210/jc.2018-01865
17. Tavita N, Greaves RF. Systematic review of serum steroid reference intervals developed using mass spectrometry. *Clin Biochem*. 2017;50(18):1260-1274. doi:10.1016/j.clinbiochem.2017.07.002
18. Ueland GÅ, Methlie P, Øksnes M, et al. The Short Cosyntropin Test Revisited: New Normal Reference Range Using LC-MS/MS. *J Clin Endocrinol Metab*. 2018;103(4):1696-1703. doi:10.1210/jc.2017-02602

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, 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

Precautions:

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:

Minimal requirement for 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:

Overnight LOW Dose Dexamethasone Suppression Test
15 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 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, 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 for IV cannulation and blood sampling

- IV cannula, blood tubes, 2ml and 5ml syringes, 0.9% saline for IV cannula flushes etc

Observations:

On arrival: BP, pulse, weight, height

Blood glucose level via glucometer on each blood sample

Method:

1. Weigh patient and take baseline observations.
2. Calculate dexamethasone dose.
3. Collect 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 dexamethasone (as per dose section) at 23:00 on Day 1.

Sample collection:

5. Collect blood sample for cortisol at 09:00 on Day 2.
6. Remove 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.

Drug Administered:		**Dose:**	**Time:**	
Actual Time bloods taken:		**Day 1**		**Day 2**
Sample	Tube Blood Volume	0800 Day 1	2300 Administer dexamethasone	0800 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) 1.5ml (on ice)	✓		✓

Discharge:

Child must have eaten and have a normal blood glucose level. All observations should be within normal limits. If abnormal repeat as required. Review by medical personnel or fulfilment of criteria-led discharge parameters prior to discharge.

Interpretation:

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

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 can 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 can include

- CYP3A4 inhibitors that decrease dexamethasone metabolism, for example fluoxetine, cimetidine, 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:

- Low-dose dexamethasone suppression test
- 24-hour urine collection for urinary free cortisol excretion (x 2-3 samples over 2-3 days)
- Serial cortisol levels (serum or salivary) at 0900, 1800, midnight, for circadian rhythm profile (for serum cortisol measurements, intravenous cannula should be inserted at least 2 hours prior to sample collection)
- Late night salivary cortisol level collected between 2300 – 2400 (x 2-3 samples over 2-3 nights)

Late night cortisol level

There is paediatric data that shows a midnight serum cortisol value ≥4.4 mcg/dL (≥121 nmol/L) confirmed 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 shows that late night salivary cortisol samples collected at bedtime rather than midnight can reduce false positive results as the circadian rhythm's cortisol nadir is tightly entrained to sleep onset.

REFERENCES

1. Batista DL, Riar J, Keil M, et al. Diagnostic Test for Children Who Are Referred for the Investigation of Cushing Syndrome. *Pediatrics.* 2007;120;e575-e586. DOI: 10.1542/peds.2006-2402
2. Batista DL, Courcoutsakis N, Riar J, et al. Severe Obesity Confounds the Interpretation of Low-Dose Dexamethasone Test Combine with the Administration of Ovine Corticotrophin-Releasing Hormone in Chilhood Cushing Syndrome. *J Clin Endocrinol Metab.* 2008;93(11):4323-4330
3. Dogra P, Vijayashankar NP. Dexamethasone Suppression Test. [Updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542317/
4. Fleseriu M, Auchus R, Bancos I, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol*. 2021;9(12):847-875. doi:10.1016/S2213-8587(21)00235-7
5. Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2008;93(5):1526-1540. doi:10.1210/jc.2008-0125
6. Savage MO, Chan LF, Grossman AB, Storr HL. Work-up and management of paediatric Cushing's syndrome. *Curr Opin Endocrinol Diabetes Obes*. 2008;15(4):346-351. doi:10.1097/MED.0b013e328305082f
7. Stratakis CA, Sarlis N, Kirschner LS, et al. Paradoxical Response to Dexamethasone in the Diagnosis of Primary Pigmented Nodular Adrenocortical Disease. *Ann Intern Med.* 1999;131:585-591
8. Stratakis CA. Cushing syndrome in pediatrics. Endocrinol Metab Clin North Am. 2012 Dec;41(4):793-803. doi: 10.1016/j.ecl.2012.08.002. Epub 2012 Sep 27. PMID: 23099271; PMCID: PMC3594781.
9. Stratakis CA. Diagnosis and Clinical Genetics of Cushing Syndrome in Pediatrics. *Endocrinol Metab Clin North Am*. 2016;45(2):311-328. doi:10.1016/j.ecl.2016.01.006

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

Indications:

Once the presence of hypercortisolism (Cushing syndrome) has been confirmed, the high dose DST is one of the subsequent investigations done to assist with identifying the cause of hypercortisolism (Cushing syndrome). The test is used to distinguish Cushing disease (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 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, 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

Precautions:

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:

Minimal requirement for 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:

Overnight HIGH Dose Dexamethasone Suppression Test
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 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, 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 for IV cannulation and blood sampling

- IV cannula, blood tubes, 2ml and 5ml syringes, 0.9% saline for IV cannula flushes etc

Observations:

On arrival: BP, pulse, weight, height

Blood glucose level via glucometer on each blood sample

Method:

1. Weigh and measure patient and take baseline observations.
2. Calculate dexamethasone dose.
3. Collect 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 dexamethasone (as per dose section) at 23:00 on Day 1.
5. Collect blood sample for cortisol at 09:00 on Day 2.
6. Remove 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:

Child must have eaten and have a normal blood glucose level. All observations should be within normal limits, if abnormal repeat as required. Review by medical personnel or fulfilment of criteria-led discharge parameters prior to discharge.

Sample collection:

Drug Administered:		**Dose:**	**Time:**	
Actual Time bloods taken:		**Day 1**		**Day 2**
Sample	Tube Blood Volume	0800 Day 1	2300 Administer dexamethasone	0800 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) 1.5ml (on ice)	✓		✓

Interpretation:

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 tumor)
Unsuppressed / suppressed < 20% from baseline (pre-dexamethasone) cortisol level	Suggests that the cause of hypercortisolism is due to an ACTH-independent cortisol producing adrenal tumours or ectopic ACTH production

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 can 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 can include

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

REFERENCES:

1. Batista DL, Riar J, Keil M, et al. Diagnostic Test for Children Who Are Referred for the Investigation of Cushing Syndrome. *Pediatrics.* 2007;120;e575-e586. DOI: 10.1542/peds.2006-2402
2. Batista DL, Courcoutsakis N, Riar J, et al. Severe Obesity Confounds the Interpretation of Low-Dose Dexamethasone Test Combine with the Administration of Ovine Corticotrophin-Releasing Hormone in Chilhood Cushing Syndrome. *J Clin Endocrinol Metab.* 2008;93(11):4323-4330
3. Dogra P, Vijayashankar NP. Dexamethasone Suppression Test. [Updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542317/
4. Fleseriu M, Auchus R, Bancos I, et al. Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol*. 2021;9(12):847-875. doi:10.1016/S2213-8587(21)00235-7
5. Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2008;93(5):1526-1540. doi:10.1210/jc.2008-0125
6. Savage MO, Chan LF, Grossman AB, Storr HL. Work-up and management of paediatric Cushing's syndrome. *Curr Opin Endocrinol Diabetes Obes*. 2008;15(4):346-351. doi:10.1097/MED.0b013e328305082f
7. Stratakis CA, Sarlis N, Kirschner LS, et al. Paradoxical Response to Dexamethasone in the Diagnosis of Primary Pigmented Nodular Adrenocortical Disease. *Ann Intern Med.* 1999;131:585-591
8. Stratakis CA. Cushing syndrome in pediatrics. Endocrinol Metab Clin North Am. 2012 Dec;41(4):793-803. doi: 10.1016/j.ecl.2012.08.002. Epub 2012 Sep 27. PMID: 23099271; PMCID: PMC3594781.
9. Stratakis CA. Diagnosis and Clinical Genetics of Cushing Syndrome in Pediatrics. *Endocrinol Metab Clin North Am*. 2016;45(2):311-328. doi:10.1016/j.ecl.2016.01.006

ORAL GLUCOSE TOLERANCE TEST (OGTT)
For Investigation of Diabetes Mellitus

Indications:

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.

Rationale:

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.

Contraindications:

Consider terminating test if fasting hyperglycaemia >10 mmol/L on glucose meter.

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. The test is invalid in the presence of intercurrent illness.

Recent surgery or trauma which may impair glucose tolerance.

Note: beta-blockers, corticosteroids, phenytoin, thiazides, oestrogens and intercurrent illness can impair glucose tolerance. Caution should be taken.

Note: stage 3b type 1 diabetes mellitus is a clinical diagnosis in a person who is multiple islet autoantibody positive, based on the presence of hyperglycaemia (random plasma glucose ≥11.1mmol/l, fasting glucose ≥7.8 mmol/L), 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 < 7.8mmol/L) or 2 type 1 diabetes (impaired glucose tolerance with 2-hour glucose 7.8-11.0mmol/L) for staging.

Expertise level:

Minimal requirement for 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 patient's 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 with an OGTT.

Normal physical activity, no intercurrent illness.

The test should be performed in the morning after a 10-16 hour overnight fast. Water is permitted.

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

Equipment:

Equipment for IV cannulation and blood sampling

- IV cannula, blood tubes, 2ml and 5ml syringes, 0.9% saline for IV cannula flushes etc

Access to hypoglycaemia treatment supplies (see Notes section below)

Observations:

On arrival: BP, pulse, weight, height

Blood glucose level via glucometer on each blood sample

Method:

1. Weigh patient and take baseline observations.
2. Calculate and measure out volume of glucose solution to be consumed (if not already pre-prepared).
3. Insert IV cannula.
4. Collect baseline (pre-stimulation) bloods and also measure glucose level on bedside/point of care glucometer.
5. Glucose drink to be consumed over **no more** than 5 minutes.
6. Emphasize patient is to be resting during the test. Water is permitted.
7. Blood samples collected at timed intervals as per table below. Glucose level to be measured on bedside/point of care glucometer at each sampling time point as well. Blood samples are timed from the moment of the first swallow, which is defined as time 0.
8. Patient to be fed before discharge. Remove IV cannula if diet and fluids are tolerated.

Discharge:

Child must have eaten and have a normal blood glucose level. All observations should be within normal limits, if abnormal repeat as required. Review by medical personnel or fulfilment of criteria-led discharge parameters prior to discharge.

Sample collection:

	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 SMO responsible for patient	+/-		+/-	+/-
Sample tubes / Minimum blood volumes	FlOx 0.5 mL		FlOx 0.5 mL	FlOx 0.5 mL

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.

	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

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

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 or 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 it used to. 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 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 oral glucose tolerance test is recommended to stage disease.

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

*Islet autoantibodies: IA2, GAD, zinc transporter 8 (ZnT8), insulin antibodies

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.

Glucose levels of ≥ 8.2 mmol/L (usually occurring at 30, 60 or 90 minutes post glucose load) in children with cystic fibrosis are associated with suboptimal weight gain so additional blood glucose monitoring with CGM should be considered. During an OGTT, when screening for CFRD, consideration should be given for measuring an additional glucose level at 1-hour (although evidence to mandate this is currently insufficient).

The North American Cystic Fibrosis Foundation Criteria classifies CF patients into subgroups based on the blood glucose levels at additional time points: 30, 60, 90 min including normal glycaemia, indeterminate glycaemia, impaired glucose tolerance, and CFRD. These criteria have not been universally adopted, and blood glucose levels at these additional timepoints are not used in the diagnosis of CFRD.

Onset of CFRD is defined as the first time a person with CF meets criteria for CFRD, even if glucose tolerance subsequently improves. ISPAD 2022 Clinical Practice Consensus Guidelines for making a diagnosis of CFRD:

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

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

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

REFERENCES:

1. American Diabetes Association Professional Practice Committee. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. *Diabetes Care*. 2022;45(Suppl 1):S17-S38. doi:10.2337/dc22-S002

2. Besser REJ, Bell KJ, Couper JJ, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Stages of type 1 diabetes in children and adolescents. *Pediatr Diabetes*. 2022;23(8):1175-1187. doi:10.1111/pedi.13410

3. Greeley SAW, Polak M, Njølstad PR, et al. ISPAD Clinical Practice Consensus Guidelines 2022: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2022;23(8):1188-1211. doi:10.1111/pedi.13426

4. Libman I, Haynes A, Lyons S, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Definition, epidemiology, and classification of diabetes in children and adolescents. *Pediatr Diabetes*. 2022;23(8):1160-1174. doi:10.1111/pedi.13454

5. Mainguy C, Bellon G, Delaup V, et al. Sensitivity and specificity of different methods for cystic fibrosis-related diabetes screening: is the oral glucose tolerance test still the standard?. *J Pediatr Endocrinol Metab*. 2017;30(1):27-35. doi:10.1515/jpem-2016-0184

6. Ode KL, Ballman M, Battezzati A, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Management of cystic fibrosis-related diabetes in children and adolescents. *Pediatr Diabetes*. 2022;23(8):1212-1228. doi:10.1111/pedi.13453

7. Prentice BJ, Jaffe A, Hameed S, Verge CF, Waters S, Widger J. Cystic fibrosis-related diabetes and lung disease: an update. *Eur Respir Rev*. 2021;30(159):200293. Published 2021 Feb 16. doi:10.1183/16000617.0293-2020

8. Prentice B, Hameed S, Verge CF, Ooi CY, Jaffe A, Widger J. Diagnosing cystic fibrosis-related diabetes: current methods and challenges. *Expert Rev Respir Med*. 2016;10(7):799-811. doi:10.1080/17476348.2016.1190646

9. Shah AS, Zeitler PS, Wong J, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Type 2 diabetes in children and adolescents. *Pediatr Diabetes*. 2022;23(7):872-902. doi:10.1111/pedi.13409

10. Wiener K. What is 75g of glucose?. *Ann Clin Biochem*. 1990;27 (Pt 4):283-284. doi:10.1177/000456329002700401

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

b) there has been resolution of disease in those with transient hyperinsulinism or in those who have undergone 'curative' surgery (partial pancreatectomy)

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 that it is preferrable to avoid a fasting study if possible due to the labour- and resource-intense nature of the study by collecting a 'critical sample' in a child (if safe) who presents with confirmed hypoglycaemia, before treatment is initiated.

Contraindications:

Recent or intercurrent illness

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 this performed in a setting where there is close monitoring throughout the test.

Expertise level:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including ability to site an IV cannula. Potentially a very hazardous test that requires very close supervision by experienced personnel.

Formulation & Dose:

Management options for hypoglycaemia during fast, 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 dextrose gel 40% (neonates)	0.5 ml / kg (200 mg / kg)	Buccal (massage into the inner cheek of the neonate)

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

Adverse reactions:

Signs and symptoms of hypoglycaemia including sweating, pallor, hunger, nausea, altered behaviour, altered level of consciousness, seizures. Cardiac arrhythmias (fatty acid oxidation disorders).

Preparation:

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

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

SMO responsible for patient to specify plans for fasting (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. Fasting commencement time should be chosen so that any hypoglycaemia is likely to occur during the day when the ward is fully staffed.

Suggested times for commencement of fasting & length of fasting based on age

Age	Fasting commencement time	Maximum length of fast
< 6 months	0400	8 hours
6 – 8 months	0000 (midnight)	12 hours
8 – 12 months	2000 the night prior	16 hours
1 – 2 years	2000 the night prior	18 hours

2 – 7 years	1600 – 1800 the evening prior	20 – 24 hours
> 7 years	1600 the afternoon prior	24 hours

Consultation with 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 SMO during the planning phase of the test is highly recommended as they will likely specify that collection of glucose and lactate 2 hours after treatment with glucose and food is required.

SMO responsible for patient to determine if a glucagon stimulation test or OGTT is indicated at the end of the fasting study.

Equipment:

Equipment for IV cannulation, blood collection and urine collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes, urine pottle, access to ice (to send samples on ice if required) etc

Bedside glucometer that is 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:

Temperature, BP, HR, RR at baseline and then every 30 minutes throughout the test

Method:

1. Ensure the appropriate steps from 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 patient, calculate doses of glucose for management of hypoglycaemia.
3. Insert IV cannula from time of last meal (for short fasts) or from 0600 – 0800 (for longer fasts). In children with a history of severe symptomatic hypoglycaemia, having 2 IV lines is 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 SMO responsible for the patient about how many IV lines 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 blood glucose level hourly (using a glucometer) while the blood glucose level is above 4.0 mmol/L.
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 table.

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 (need rapid result to confirm accuracy of glucometer result)
2.6 mmol/L and below (confirm on 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 (need rapid result to confirm accuracy of glucometer result) Collect blood and urine samples (see sample collection section)
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), and consider checking a 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 appropriately mobilised during hypoglycaemia / following glucagon stimulation.

If this is the case:

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

2. Administer glucagon:

Dose: 30 micrograms / kg (max 1 milligram)

Route: Slow intravenous push

3. Measure glucose (bedside glucometer + lab) and insulin levels at:

10 Min, 20 Min, 30 Min 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 10% glucose IV bolus 2ml/kg.

Discharge:

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. If abnormal, repeat as required. The child should stay in the ward for 2 – 4 hours following completion of the fasting test (or a time determined by the doctor). Discussion with +/- review by medical personnel prior to discharge.

Sample collection:

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)
Plasma amino acids
Ammonia (send to lab on ice, must arrive within 30 minutes)
CK, LFTs, lipids, urate
Pyruvate - if lactate high / inborn error of metabolism suspected
URINE (first urine void post hypoglycaemia)
Metabolic screen (organic acids, amino acids)
Ketones

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

Blood and urine samples can be collected when in a state of normoglycaemia for some investigations:

- Early morning cortisol + ACTH (+/- very long chain fatty acids if suspect Addison's disease in a boy, to assess for adrenoleukodystrophy)
- Lactate, ammonia, transferrin isoforms
- Acylcarnitine profile
- Urine organic acids
- Growth hormone stimulation test
- DNA – for storage or to send away for a specific gene panel

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 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.

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
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. The table below includes some of the differential diagnoses to consider.

KETOTIC HYPOGLYCAEMIA
Endocrine Causes • Adrenal insufficiency (cortisol deficiency) • Growth hormone deficiency • Hypopituitarism (ACTH &/or GH deficiency)
Metabolic Causes • Idiopathic ketotic hypoglycaemia • Glycogen storage disorders (GSDs) • Gluconeogenic defects • Other inborn errors of metabolism (IEMs)
NON-KETOTIC HYPOGLYCAEMIA
Endocrine Causes • Hyperinsulinism
Metabolic Causes • Fatty acid oxidation disorders (FAODs) • Ketogenesis defects • Congenital disorders of glycosylation

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 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.

REFERENCES

1. Bappal B, Mula-Abed WA. Evaluation of diagnostic fasting in the investigation of hypoglycemia in children omani experienc. *Oman Med J*. 2007;22(3):36-41.
2. Casertano A, Rossi A, Fecarotta S, et al. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. *Front Endocrinol (Lausanne)*. 2021;12:684011. Published 2021 Aug 2. doi:10.3389/fendo.2021.684011
3. Crofton PM, Midgley PC. Cortisol and growth hormone responses to spontaneous hypoglycaemia in infants and children. *Arch Dis Child*. 2004;89(5):472-478. doi:10.1136/adc.2003.033431
4. Graves LE, Stewart K, Ambler GR, Bhattacharya K, Srinivasan S. Investigating paediatric hypoglycaemia: Dynamic studies at a tertiary paediatric hospital. *J Paediatr Child Health*. 2021;57(6):888-893. doi:10.1111/jpc.15349
5. Hawkes CP, Grimberg A, Dzata VE, De Leon DD. Adding Glucagon-Stimulated GH Testing to the Diagnostic Fast Increases the Detection of GH-Sufficient Children. *Horm Res Paediatr*. 2016;85(4):265-272. doi:10.1159/000444678
6. Hussain K, Hindmarsh P, Aynsley-Green A. Neonates with symptomatic hyperinsulinemic hypoglycemia generate inappropriately low serum cortisol counterregulatory hormonal responses. *J Clin Endocrinol Metab*. 2003;88(9):4342-4347. doi:10.1210/jc.2003-030135
7. Kaplowitz P, Sekizkardes H. Clinical and laboratory characteristics and follow up of 62 cases of ketotic hypoglycemia: a retrospective study. *Int J Pediatr Endocrinol*. 2019;2019:3. doi:10.1186/s13633-019-0066-9
8. Kelly A, Tang R, Becker S, Stanley CA. Poor specificity of low growth hormone and cortisol levels during fasting hypoglycemia for the diagnoses of growth hormone deficiency and adrenal insufficiency. *Pediatrics*. 2008;122(3):e522-e528. doi:10.1542/peds.2008-0806
9. Maiorana A, Lepri FR, Novelli A, Dionisi-Vici C. Hypoglycaemia Metabolic Gene Panel Testing. *Front Endocrinol (Lausanne)*. 2022;13:826167. Published 2022 Mar 29. doi:10.3389/fendo.2022.826167
10. Sprague JE, Arbeláez AM. Glucose counterregulatory responses to hypoglycemia. *Pediatr Endocrinol Rev*. 2011;9(1):463-475.
11. Sreekantam S, Preece MA, Vijay S, Raiman J, Santra S. How to use a controlled fast to investigate hypoglycaemia. *Arch Dis Child Educ Pract Ed*. 2017;102(1):28-36. doi:10.1136/archdischild-2015-308702

WATER DEPRIVATION TEST

Indications:

For the investigation of polyuria-polydipsia syndrome. 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 to promote reabsorption of water via aquaporin channels which leads to declining urine volumes, increasing urine osmolality, and prevention of further increase in plasma osmolality, 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.

Contraindications:

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

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, electrolyte disturbance, and ensure diagnostic samples are collected appropriately.

Preparation:

Prior to proceeding with a WDT, if it is unclear from 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, check a random, baseline copeptin level. If the copeptin level is elevated > 21.4 pmol/L, this is consistent with AVP resistance and a WDT or arginine-stimulated copeptin test will not be required.

Exclude other causes of polyuria/polydipsia such as hyperglycaemia (diabetes mellitus), hypercalcaemia, hypokalaemia, hypothyroidism, hypoadrenalism, UTI, chronic renal failure, administration of large volumes of normal saline (sodium-induced polyuria), 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 SMO 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 run for

Inform your hospital laboratory of the date/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.

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.

Formulation & Dose:

Formulation	Dose	Route
Desmopressin injection 4 mcg/ml (1ml ampoule)	0.1 micrograms (< 2 years) 0.2 micrograms (2 – 7years) 0.3 micrograms (8 – 14 years) 0.4 micrograms (>14 years)	Intravenous
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 for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

Urine pan/container/urinal, measuring equipment for urine + urine pottles

Digital weighing scales (to weigh patient)

Desmopressin

Worksheet

Observations:

BP and HR hourly

BGL with every blood test.

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

Method:

Prior to starting the WDT, liaise with the SMO responsible for the patient and complete the 'Water Deprivation Test – Preparation Worksheet'.

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'. 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 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, administer desmopressin or cease 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 0800):

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)

0800: start WDT (= Time 0)

Measurements and sample collections as per 'Water Deprivation Test – Worksheet for use during testing'.

Complete the following table:

Location where WDT will take place (circle location)	Inpatient / Day Unit
Date/time that patient should arrive (consider admitting the night prior vs coming in the morning of test)	
Date/time that patient should stop eating food	
Date/time that patient should stop drinking fluids*	
Date/time that the WDT will commence (often 0800)	
Date/time that period of water deprivation should cease (if 'Termination Criteria' are not met prior)**	
Staff to be present for the duration of the WDT (circle which staff are required)	Nurse / nurse specialist / nurse practitioner / RMO / SMO

*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 0800 following consumption of breakfast (with no more than 100 – 150ml fluid consumed). Where less rapid dehydration is anticipated, for example, 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

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

Termination Criteria:

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

Plasma osmolality	> 300 mOsm/kg
Urine osmolality	> 750mOsm/kg (or > 500mOsm/kg in infants) OR Consistently < 30 mOsm/kg between 3 consecutive samples
Weight	> 5% loss from baseline weight

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, administer desmopressin or cease 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.

Record sheet:

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

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

*For blood samples with plasma sodium ≥ 147 mmol/L, add on a copeptin level. The rationale for this being that hyperosmolality is a stimulus for both copeptin and AVP secretion. AVP and copeptin are peptides derived from the same preprohormone, ‘pre-pro-vasopressin', and are secreted in equimolar amounts in response to similar physiological stimuli such as osmotic stimulation. 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. Copeptin measurement takes less than 2hours once on the analyser but results may take several days to come back (depending on whether the test is performed onsite or sent away to another laboratory), however they can still be of great assistance in the diagnostic process for differentiating between central/cranial diabetes insipidus and primary polydipsia. See interpretation section for further details.

**Record volume of all urine passed and reserve a 10ml 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 200ml 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).

Sample collection:

Plasma sodium, glucose, potassium, urea, creatinine, osmolality, copeptin	2ml Plain, PST (Li hep with gel), SST, Li hep
Urine osmolality	1 - 10ml in the urine collection pottle/tube specific for your laboratory

Interpretation:

	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 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 unsatisfactory test, partial DI, chronic renal failure or diuretic administration		

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:

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.

Desmopressin route of administration note

Oral administration of desmopressin is NOT the preferred route of administration due to unpredictable absorption via this route of administration.

If the patient has nasal congestion or it is anticipated that intranasal administration will be challenging, then intravenous, intramuscular, or subcutaneous route is recommended.

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

As 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

*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.

REFERENCES

1. Armstrong LE, Johnson EC. Water Intake, Water Balance, and the Elusive Daily Water Requirement. *Nutrients*. 2018;10(12):1928. Published 2018 Dec 5. doi:10.3390/nu10121928
2. Bakhtiani P, Geffner ME. Diagnosing DI (The Water Deprivation Test). In: Alter CA, ed. *Diabetes Insipidus in Children*. Springer, Cham; 2021:9-22. doi:10.1007/978-3-030-83248-3_2.
3. Berton AM, Gatti F, Penner F, et al. Early Copeptin Determination Allows Prompt Diagnosis of Post-Neurosurgical Central Diabetes Insipidus. *Neuroendocrinology*. 2020;110(6):525-534. doi:10.1159/000503145
1. Binder G, Weber K, Peter A, Schweizer R. Arginine-stimulated copeptin in children and adolescents. *Clin Endocrinol (Oxf)*. 2023;98(4):548-553. doi:10.1111/cen.14880
2. Bitencourt L, Fischer BL, de Oliveira Campos JL, et al. The usefulness of copeptin for the diagnosis of nephrogenic diabetes insipidus in infancy: a case report. *J Pediatr Endocrinol Metab*. 2021;34(11):1475-1479. Published 2021 Jul 22. doi:10.1515/jpem-2021-0296
3. Bonnet L, Marquant E, Fromonot J, et al. Copeptin assays in children for the differential diagnosis of polyuria-polydipsia syndrome and reference levels in hospitalized children. *Clin Endocrinol (Oxf)*. 2022;96(1):47-53. doi:10.1111/cen.14620
4. Christ-Crain M. Vasopressin and Copeptin in health and disease. *Rev Endocr Metab Disord*. 2019;20(3):283-294. doi:10.1007/s11154-019-09509-9
5. Christ-Crain M. EJE AWARD 2019: New diagnostic approaches for patients with polyuria polydipsia syndrome. *Eur J Endocrinol*. 2019;181(1):R11-R21. doi:10.1530/EJE-19-0163
6. Christ-Crain M, Fenske WK. Copeptin in the differential diagnosis of hypotonic polyuria. *J Endocrinol Invest*. 2020;43(1):21-30. doi:10.1007/s40618-019-01087-6
7. Christ-Crain M. Diabetes Insipidus: New Concepts for Diagnosis. *Neuroendocrinology*. 2020;110(9-10):859-867. doi:10.1159/000505548
8. Christ-Crain M, Winzeler B, Refardt J. Diagnosis and management of diabetes insipidus for the internist: an update. *J Intern Med*. 2021;290(1):73-87. doi:10.1111/joim.13261
9. Christ-Crain M, Refardt J, Winzeler B. Approach to the Patient: "Utility of the Copeptin Assay". *J Clin Endocrinol Metab*. 2022;107(6):1727-1738. doi:10.1210/clinem/dgac070
10. de Fost M, Oussaada SM, Endert E, et al. The water deprivation test and a potential role for the arginine vasopressin precursor copeptin to differentiate diabetes insipidus from primary polydipsia. *Endocr Connect*. 2015;4(2):86-91. doi:10.1530/EC-14-0113
11. Evers KS, Wellmann S. Arginine Vasopressin and Copeptin in Perinatology. *Front Pediatr*. 2016;4:75. Published 2016 Aug 2. doi:10.3389/fped.2016.00075
12. Fenske W, Quinkler M, Lorenz D, et al. Copeptin in the differential diagnosis of the polydipsia-polyuria syndrome--revisiting the direct and indirect water deprivation tests. *J Clin Endocrinol Metab*. 2011;96(5):1506-1515. doi:10.1210/jc.2010-2345
13. Fenske W, Refardt J, Chifu I, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. *N Engl J Med*. 2018;379(5):428-439. doi:10.1056/NEJMoa1803760

14. Frasier SD, Kutnik LA, Schmidt RT, Smith FG Jr. A water deprivation test for the diagnosis of diabetes insipidus in children. *Am J Dis Child*. 1967;114(2):157-160. doi:10.1001/archpedi.1967.02090230087009
15. Garibaldi LR, Gurtunca N, March C, et al. The Arginine Stimulation Test Allows Rapid Diagnosis of Central Diabetes Insipidus in Children. *J Endocrine Soc.* 2021:5 (Issue Supplement_1):A635-A636. doi:10.1210/jendoso/bvab048
16. Garibaldi L, March C, McPhaul M, et al. PMON180 Extremely Elevated Serum Copeptin Concentrations Following Venipuncture Occur in Approximately 5% of Children and May Affect Proper Interpretation of Copeptin Measurement. *J Endocrine Soc.* 2022:6 (Issue Supplement_1): A549-A550. doi:10.1210/jendso/bvac150.1142
17. Grandone A, Marzuillo P, Patti G, Perrotta S, Maghnie M. Changing the diagnostic approach to diabetes insipidus: role of copeptin. *Ann Transl Med*. 2019;7(Suppl 8):S285. doi:10.21037/atm.2019.11.80
18. Gubbi S, Hannah-Shmouni F, Koch CA, et al. Diagnostic Testing for Diabetes Insipidus. [Updated 2022 Nov 28]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537591/
19. Hasegawa Y. The relationship between antidiuretic hormone and plasma or urine osmolalities during water restriction test and hypertonic saline loading test in normal children--a change in the apparent tubular response to AVP during these two tests. *Endocrinol Jpn*. 1991;38(5):451-456. doi:10.1507/endocrj1954.38.451
20. Jain V, Ravindranath A. Diabetes insipidus in children. *J Pediatr Endocrinol Metab*. 2016;29(1):39-45. doi:10.1515/jpem-2014-0518
21. Kamel KS, Halperin ML. Use of Urine Electrolytes and Urine Osmolality in the Clinical Diagnosis of Fluid, Electrolytes, and Acid-Base Disorders. *Kidney Int Rep*. 2021;6(5):1211-1224. Published 2021 Feb 13. doi:10.1016/j.ekir.2021.02.003
22. March CA, Sastry S, McPhaul MJ, Wheeler SE, Garibaldi L. Combined Arginine and Insulin Stimulation Elicits a Robust and Consistent Copeptin Response in Short Children. *Horm Res Paediatr*. 2023;96(4):395-403. doi:10.1159/000528661
23. Mu D, Ma C, Cheng J, Zou Y, Qiu L, Cheng X. Copeptin in fluid disorders and stress. *Clin Chim Acta*. 2022;529:46-60. doi:10.1016/j.cca.2022.02.002
24. Nigro N, Grossmann M, Chiang C, Inder WJ. Polyuria-polydipsia syndrome: a diagnostic challenge. *Intern Med J*. 2018;48(3):244-253. doi:10.1111/imj.13627
25. Polacek E, Vocel J, Neugebauerova L, Sebkova M, Vechetova E. The Osmotic Concentrating Ability in Healthy Infants and Children. *Arch Dis Child*. 1965;40(211):291-295. doi:10.1136/adc.40.211.291
26. Qureshi S, Galiveeti S, Bichet DG, Roth J. Diabetes insipidus: celebrating a century of vasopressin therapy. *Endocrinology*. 2014;155(12):4605-4621. doi:10.1210/en.2014-1385
27. Refardt J, Christ-Crain M. Copeptin-based diagnosis of diabetes insipidus. *Swiss Med Wkly*. 2020;150:w20237. Published 2020 May 6. doi:10.4414/smw.2020.20237

28. Refardt J, Winzeler B, Christ-Crain M. Diabetes Insipidus: An Update. *Endocrinol Metab Clin North Am*. 2020;49(3):517-531. doi:10.1016/j.ecl.2020.05.012
29. Refardt J. Diagnosis and differential diagnosis of diabetes insipidus: Update. *Best Pract Res Clin Endocrinol Metab*. 2020;34(5):101398. doi:10.1016/j.beem.2020.101398
30. Rothermel J, Kulle A, Holterhus PM, et al. Copeptin in obese children and adolescents: relationships to body mass index, cortisol and gender. *Clin Endocrinol.* 2016:85(6):868-873. doi:10.1111/cen.13234
31. Sailer CO, Refardt J, Blum CA, et al. Validity of different copeptin assays in the differential diagnosis of the polyuria-polydipsia syndrome. *Sci Rep*. 2021;11(1):10104. Published 2021 May 12. doi:10.1038/s41598-021-89505-9
32. Sjöström A, Bartuseviciene I, Höybye C. Simplified and improved fluid deprivation test for diagnosing diabetes insipidus. *Eur J Endocrinol*. 2021;184(1):123-131. doi:10.1530/EJE-20-0759
33. Timper K, Fenske W, Kühn F, et al. Diagnostic Accuracy of Copeptin in the Differential Diagnosis of the Polyuria-polydipsia Syndrome: A Prospective Multicenter Study. *J Clin Endocrinol Metab*. 2015;100(6):2268-2274. doi:10.1210/jc.2014-4507
34. Tuli G, Tessaris D, Einaudi S, Matarazzo P, De Sanctis L. Copeptin role in polyuria-polydipsia syndrome differential diagnosis and reference range in paediatric age. *Clin Endocrinol (Oxf)*. 2018;88(6):873-879. doi:10.1111/cen.13583
35. Vaz de Castro PAS, Bitencourt L, de Oliveira Campos JL, et al. Nephrogenic diabetes insipidus: a comprehensive overview. *J Pediatr Endocrinol Metab*. 2022;35(4):421-434. Published 2022 Feb 11. doi:10.1515/jpem-2021-0566
36. Weiner A, Vuguin P. Diabetes Insipidus. *Pediatr Rev*. 2020;41(2):96-99. doi:10.1542/pir.2018-0337
37. Winzeler B, Cesana-Nigro N, Refardt J, et al. Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study. *Lancet*. 2019;394(10198):587-595. doi:10.1016/S0140-6736(19)31255-3
38. Wong LM, Man SS. Water deprivation test in children with polyuria. *J Pediatr Endocrinol Metab*. 2012;25(9-10):869-874. doi:10.1515/jpem-2012-0092

ARGININE STIMULATED COPEPTIN TEST

Indications:

To assess for the presence of Arginine Vasopressin (AVP) deficiency, and differentiate between AVP deficiency (central diabetes insipidus) and primary polydipsia, in individuals who present with polyuria-polydipsia syndrome. In individuals with AVP resistance (nephrogenic diabetes insipidus), an elevated baseline (unstimulated) copeptin level is consistent with this diagnosis and a stimulated copeptin test is not required.

Rationale:

AVP, also known as anti-diuretic hormone (ADH), is a key hormone involved in the body's water-balance system. It is synthesized in hypothalamic nuclei and stored in the posterior pituitary gland from where it is released into the circulation when the plasma/serum osmolality increases above a certain threshold (around 285 mmol/kg). AVP acts on the collecting tubules in the kidneys to promote reabsorption of water via aquaporin-2 channels, which in turn leads to production of smaller volumes of more concentrated urine (antidiuresis) and prevents continued increase in plasma/serum osmolality (hyperosmolar state/dehydration).

Diabetes insipidus occurs when there is a deficiency in AVP (central diabetes insipidus) or resistance to AVP (nephrogenic diabetes insipidus). AVP can be difficult to measure due technical difficulties, thus the water deprivation test (an indirect assessment of AVP status by measurement of urine concentrating ability during water deprivation) has been the gold standard test for assessment of individuals with polyuria-polydipsia syndrome for a number of years. This test can however be extremely challenging to carry out, particularly in the paediatric population.

Copeptin, the c-terminal part of the precursor pre-provasopressin, is secreted in equimolar amounts to AVP and therefore is a surrogate marker for AVP level. Copeptin is more stable than AVP, and the measurement of copeptin requires minimal pre-analytical preparation and is performed on an automated immunoassay with a short turnaround time. Both unstimulated and stimulated copeptin levels are becoming increasingly incorporated into polyuria-polydipsia diagnostic algorithms. Stimulation of copeptin release can be induced by either an osmolar (for example, hypertonic saline) or non-osmolar (e.g. arginine, glucagon) stimulus.

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

Certain drugs, for example, periactin, interfere with arginine stimulation

People with known allergic tendencies

Precautions:

Ensure the patient has robust intravenous access for arginine infusion

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.

Expertise level:

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

Formulation & 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)	Intravenous infusion over 30 minutes

Adverse reactions:

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Preparation:

Prior to arranging this test, check a random, unstimulated copeptin level. If this is elevated > 21.4 pmol/L, this is consistent with AVP resistance and the arginine-stimulated copeptin test will not be required

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patients with adrenal insufficiency are on appropriate glucocorticoid replacement prior to commencing test.

The consultant responsible for the patient should specify the plan for fasting based on their evaluation of the severity of the patient's condition and the likely risk to them undergoing this test. The aim is to start the arginine infusion at 0800am.

Fluid restriction

- High risk patients (those at high risk of dehydration if fluid is restricted for 8 hours prior to commencing the test, such as infants, young children, children with suspected diabetes insipidus)
    - May drink freely until 0800am on the day of the test (that is, until the arginine infusion commences)
- Low risk patients (those at low risk of dehydration if fluid is restricted for 8 hours prior to commencing the test, such as older children who don't usually drink overnight)
    - No fluid from midnight the night prior to the test (that is, no fluids for 8 hours prior to commencement of arginine infusion)

Food restriction

- No food from midnight the night prior to the test (that is, no food for 8 hours prior to commencement of the arginine infusion) if it is safe to do so
- In infants, young children, children prone to hypoglycaemia, the fasting period may be as short as 2 hours

If the patient is usually on desmopressin (DDAVP), discontinue this at least 24 hours prior to the test. Depending on the age of the child and the anticipated risk of dehydration, if it is not safe to do this at home, the child should be admitted to hospital at least 24 hours prior to the commencement of the test so that discontinuation of DDAVP can be done in the inpatient setting where there is access to appropriate monitoring and IV/NG/PEG fluids if 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 request form.

Equipment:

Equipment for IV cannulation + blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulant – arginine

Observations:

Temperature, BP, HR, RR at baseline and then at each blood sampling timepoint during the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Aim to start arginine infusion at 0800am.
2. Document any medication(s) the patient is on, and for each medication, record the dose and date/time of the last dose (if on DDAVP, the last dose needs to have been at least 24 hours prior to commencement of arginine infusion).
3. Weigh patient, calculate arginine dose and take baseline observations.

4. 30 minutes prior to commencing arginine infusion, insert IV cannula and ensure patient is settled in a supine position.
5. Take baseline (pre-stimulation) blood samples.
6. Administer 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 0.9% saline prior to taking the 30 minute blood sample.
7. Blood sampling as below. If performed as part of a combined pituitary test, see combined protocol.
8. 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 hypo screen (if indicated and safe to do so) and then treat the hypo as per your local unit's hypoglycaemia management guideline.
9. No food or water until the test is completed.

Sample collection:

	Baseline	**Administer arginine**	**Minutes post *commencement* of arginine infusion**				
TEST	-1 Min		30 Min	45 Min	60 Min	90 Min	120 Min
Copeptin	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓
Electrolytes and plasma osmolality	✓		✓	✓	✓	✓	✓
Urine osmolality and urine volume	✓						✓
Other tests e.g. electrolytes, creatinine as per consultant responsible for patient	✓						
Weight, BP, HR	✓		✓		✓	✓	✓
Sample Tubes / Minimum Blood Volumes							

Interpretation:

Unstimulated copeptin level > 21.4 pmol/L is consistent with AVP resistance

- 100% sensitivity and specificity for diagnosing AVP resistance (Christ-Crain et al)

- Bonnet et al: 3 of 278 children aged 2 months to 18 years old (40 children with polyuria-polydipsia syndrome, 238 controls) were diagnosed with AVP resistance and all of them had copeptin > 30 pmol/L.

Arginine-stimulated copeptin level < 3.8 pmol/L at 60 minutes post-commencement of arginine infusion is consistent with AVP deficiency

- Sensitivity 93%, specificity 92% for differentiating between AVP deficiency and PP. Sensitivity 93%, specificity 80% for differentiating between partial AVP deficiency and PP (adult cohorts)
- Bonnet et al: 21 of 278 children aged 2 months to 18 years old (40 children with polyuria-polydipsia syndrome, 238 controls) were diagnosed with AVP deficiency. Their median copeptin level was 1.7 pmol/L compared to 5.5 pmol/L in the primary polydipsia group (p-value < 0.001). Copeptin < 3.53 pmol/L had a sensitivity of 100% and specificity of 87.4% for diagnosing AVP deficiency. Copeptin < 1.07 pmol/L had a sensitivity of 28.6% and specificity of 100% for diagnosing AVP 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.

Copeptin

The copeptin levels stated in the ‘Interpretation’ section pertain to copeptin measurements performed using a manual sandwich immunoluminometric assay (LIA) or automated immunofluorescent assay (KRYPTOR platform)

The stimulated copeptin threshold of 3.8 pmol/L is still awaiting validation (RCT currently underway)*

In study using arginine stimulated copeptin test, copeptin levels were taken at baseline, 30, 45, 60, 90 and 120 minutes. Arginine infusion started at 0800 after an overnight fast of 8h and fluid restriction of 2h in adults (children were allowed to drink water until test start); patients on DDAVP discontinued their medication at least 24h prior. 30 min before test start patients were settled in supine position and IVL placed. Arginine (L-arginine-hydrochloride 21%) at a dose of 0.5g/kg diluted in 500 ml of normal saline infused over 30min. BP + HR measured at the same timepoints as bloods. At baseline and end of test (120min) routine lab measurements for plasma and urine samples were done and glucose also measured at each timepoint. No association seen between GH deficiency and maximum copeptin concentrations achieved by children.

Stimuli of copeptin release include increase in plasma osmolality, decrease in arterial blood volume and pressure (volume depletion), somatic stress (seen in all states of serious illness), nausea, vomiting, physical exercise.

REFERENCES

1. Armstrong LE, Johnson EC. Water Intake, Water Balance, and the Elusive Daily Water Requirement. *Nutrients*. 2018;10(12):1928. Published 2018 Dec 5. doi:10.3390/nu10121928
2. Bakhtiani P, Geffner ME. Diagnosing DI (The Water Deprivation Test). In: Alter CA, ed. *Diabetes Insipidus in Children*. Springer, Cham; 2021:9-22. doi:10.1007/978-3-030-83248-3_2.
3. Berton AM, Gatti F, Penner F, et al. Early Copeptin Determination Allows Prompt Diagnosis of Post-Neurosurgical Central Diabetes Insipidus. *Neuroendocrinology*. 2020;110(6):525-534. doi:10.1159/000503145
4. Binder G, Weber K, Peter A, Schweizer R. Arginine-stimulated copeptin in children and adolescents. *Clin Endocrinol (Oxf)*. 2023;98(4):548-553. doi:10.1111/cen.14880
5. Bitencourt L, Fischer BL, de Oliveira Campos JL, et al. The usefulness of copeptin for the diagnosis of nephrogenic diabetes insipidus in infancy: a case report. *J Pediatr Endocrinol Metab*. 2021;34(11):1475-1479. Published 2021 Jul 22. doi:10.1515/jpem-2021-0296
6. Bonnet L, Marquant E, Fromonot J, et al. Copeptin assays in children for the differential diagnosis of polyuria-polydipsia syndrome and reference levels in hospitalized children. *Clin Endocrinol (Oxf)*. 2022;96(1):47-53. doi:10.1111/cen.14620
7. Christ-Crain M. Vasopressin and Copeptin in health and disease. *Rev Endocr Metab Disord*. 2019;20(3):283-294. doi:10.1007/s11154-019-09509-9
8. Christ-Crain M. EJE AWARD 2019: New diagnostic approaches for patients with polyuria polydipsia syndrome. *Eur J Endocrinol*. 2019;181(1):R11-R21. doi:10.1530/EJE-19-0163
9. Christ-Crain M, Fenske WK. Copeptin in the differential diagnosis of hypotonic polyuria. *J Endocrinol Invest*. 2020;43(1):21-30. doi:10.1007/s40618-019-01087-6
10. Christ-Crain M. Diabetes Insipidus: New Concepts for Diagnosis. *Neuroendocrinology*. 2020;110(9-10):859-867. doi:10.1159/000505548
11. Christ-Crain M, Winzeler B, Refardt J. Diagnosis and management of diabetes insipidus for the internist: an update. *J Intern Med*. 2021;290(1):73-87. doi:10.1111/joim.13261
12. Christ-Crain M, Refardt J, Winzeler B. Approach to the Patient: "Utility of the Copeptin Assay". *J Clin Endocrinol Metab*. 2022;107(6):1727-1738. doi:10.1210/clinem/dgac070
13. de Fost M, Oussaada SM, Endert E, et al. The water deprivation test and a potential role for the arginine vasopressin precursor copeptin to differentiate diabetes insipidus from primary polydipsia. *Endocr Connect*. 2015;4(2):86-91. doi:10.1530/EC-14-0113
14. Evers KS, Wellmann S. Arginine Vasopressin and Copeptin in Perinatology. *Front Pediatr*. 2016;4:75. Published 2016 Aug 2. doi:10.3389/fped.2016.00075
15. Fenske W, Quinkler M, Lorenz D, et al. Copeptin in the differential diagnosis of the polydipsia-polyuria syndrome--revisiting the direct and indirect water deprivation tests. *J Clin Endocrinol Metab*. 2011;96(5):1506-1515. doi:10.1210/jc.2010-2345

16. Fenske W, Refardt J, Chifu I, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. *N Engl J Med*. 2018;379(5):428-439. doi:10.1056/NEJMoa1803760
17. Frasier SD, Kutnik LA, Schmidt RT, Smith FG Jr. A water deprivation test for the diagnosis of diabetes insipidus in children. *Am J Dis Child*. 1967;114(2):157-160. doi:10.1001/archpedi.1967.02090230087009
18. Garibaldi LR, Gurtunca N, March C, et al. The Arginine Stimulation Test Allows Rapid Diagnosis of Central Diabetes Insipidus in Children. *J Endocrine Soc.* 2021:5 (Issue Supplement_1):A635-A636. doi:10.1210/jendoso/bvab048
19. Garibaldi L, March C, McPhaul M, et al. PMON180 Extremely Elevated Serum Copeptin Concentrations Following Venipuncture Occur in Approximately 5% of Children and May Affect Proper Interpretation of Copeptin Measurement. *J Endocrine Soc.* 2022:6 (Issue Supplement_1): A549-A550. doi:10.1210/jendso/bvac150.1142
20. Grandone A, Marzuillo P, Patti G, Perrotta S, Maghnie M. Changing the diagnostic approach to diabetes insipidus: role of copeptin. *Ann Transl Med*. 2019;7(Suppl 8):S285. doi:10.21037/atm.2019.11.80
21. Gubbi S, Hannah-Shmouni F, Koch CA, et al. Diagnostic Testing for Diabetes Insipidus. [Updated 2022 Nov 28]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537591/
22. Hasegawa Y. The relationship between antidiuretic hormone and plasma or urine osmolalities during water restriction test and hypertonic saline loading test in normal children--a change in the apparent tubular response to AVP during these two tests. *Endocrinol Jpn*. 1991;38(5):451-456. doi:10.1507/endocrj1954.38.451
23. Jain V, Ravindranath A. Diabetes insipidus in children. *J Pediatr Endocrinol Metab*. 2016;29(1):39-45. doi:10.1515/jpem-2014-0518
24. Kamel KS, Halperin ML. Use of Urine Electrolytes and Urine Osmolality in the Clinical Diagnosis of Fluid, Electrolytes, and Acid-Base Disorders. *Kidney Int Rep*. 2021;6(5):1211-1224. Published 2021 Feb 13. doi:10.1016/j.ekir.2021.02.003
25. March CA, Sastry S, McPhaul MJ, Wheeler SE, Garibaldi L. Combined Arginine and Insulin Stimulation Elicits a Robust and Consistent Copeptin Response in Short Children. *Horm Res Paediatr*. 2023;96(4):395-403. doi:10.1159/000528661
26. Mu D, Ma C, Cheng J, Zou Y, Qiu L, Cheng X. Copeptin in fluid disorders and stress. *Clin Chim Acta*. 2022;529:46-60. doi:10.1016/j.cca.2022.02.002
27. Nigro N, Grossmann M, Chiang C, Inder WJ. Polyuria-polydipsia syndrome: a diagnostic challenge. *Intern Med J*. 2018;48(3):244-253. doi:10.1111/imj.13627
28. Polacek E, Vocel J, Neugebauerova L, Sebkova M, Vechetova E. The Osmotic Concentrating Ability in Healthy Infants and Children. *Arch Dis Child*. 1965;40(211):291-295. doi:10.1136/adc.40.211.291
29. Qureshi S, Galiveeti S, Bichet DG, Roth J. Diabetes insipidus: celebrating a century of vasopressin therapy. *Endocrinology*. 2014;155(12):4605-4621. doi:10.1210/en.2014-1385

30. Refardt J, Christ-Crain M. Copeptin-based diagnosis of diabetes insipidus. *Swiss Med Wkly*. 2020;150:w20237. Published 2020 May 6. doi:10.4414/smw.2020.20237
31. Refardt J, Winzeler B, Christ-Crain M. Diabetes Insipidus: An Update. *Endocrinol Metab Clin North Am*. 2020;49(3):517-531. doi:10.1016/j.ecl.2020.05.012
32. Refardt J. Diagnosis and differential diagnosis of diabetes insipidus: Update. *Best Pract Res Clin Endocrinol Metab*. 2020;34(5):101398. doi:10.1016/j.beem.2020.101398
33. Rothermel J, Kulle A, Holterhus PM, et al. Copeptin in obese children and adolescents: relationships to body mass index, cortisol and gender. *Clin Endocrinol.* 2016:85(6):868-873. doi:10.1111/cen.13234
34. Sailer CO, Refardt J, Blum CA, et al. Validity of different copeptin assays in the differential diagnosis of the polyuria-polydipsia syndrome. *Sci Rep*. 2021;11(1):10104. Published 2021 May 12. doi:10.1038/s41598-021-89505-9
35. Sjöström A, Bartuseviciene I, Höybye C. Simplified and improved fluid deprivation test for diagnosing diabetes insipidus. *Eur J Endocrinol*. 2021;184(1):123-131. doi:10.1530/EJE-20-0759
36. Timper K, Fenske W, Kühn F, et al. Diagnostic Accuracy of Copeptin in the Differential Diagnosis of the Polyuria-polydipsia Syndrome: A Prospective Multicenter Study. *J Clin Endocrinol Metab*. 2015;100(6):2268-2274. doi:10.1210/jc.2014-4507
37. Tuli G, Tessaris D, Einaudi S, Matarazzo P, De Sanctis L. Copeptin role in polyuria-polydipsia syndrome differential diagnosis and reference range in paediatric age. *Clin Endocrinol (Oxf)*. 2018;88(6):873-879. doi:10.1111/cen.13583
38. Vaz de Castro PAS, Bitencourt L, de Oliveira Campos JL, et al. Nephrogenic diabetes insipidus: a comprehensive overview. *J Pediatr Endocrinol Metab*. 2022;35(4):421-434. Published 2022 Feb 11. doi:10.1515/jpem-2021-0566
39. Weiner A, Vuguin P. Diabetes Insipidus. *Pediatr Rev*. 2020;41(2):96-99. doi:10.1542/pir.2018-0337
40. Winzeler B, Cesana-Nigro N, Refardt J, et al. Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study. *Lancet*. 2019;394(10198):587-595. doi:10.1016/S0140-6736(19)31255-3
41. Wong LM, Man SS. Water deprivation test in children with polyuria. *J Pediatr Endocrinol Metab*. 2012;25(9-10):869-874. doi:10.1515/jpem-2012-0092

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

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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 48hours, 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 (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

People with known allergic tendencies

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

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 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:

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

Anaphylaxis to Tetracosactrin 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:

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

GLUCAGON

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

SYNACTHEN

Formulation	Dose	Route
	0 – 6 months old 62.5 micrograms	Intravenous

Tetracosactrin (Synacthen, solution for injection) 250 mcg in 1 mL	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Glucagon

Transient nausea, flushing, vomiting for 1 – 2 minutes, abdominal pain / cramps, 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

Synacthen

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

Preparation:

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

In individuals on chronic supra-physiological doses of glucocorticoids, an appropriate weaning regime should be performed before undertaking a SST. 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 (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

This test should be performed before 0900 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 SST), then the SST can be performed at any time of day as 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 SMO responsible for the patient about the desired timeframe post-surgery that the SST should be arranged for. Following 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 ~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 a 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, 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 request form.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	2 days, reporting next day for GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	2-3 days, reporting next day for GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, glucagon, Synacthen

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient and take baseline observations.
3. Work out and prescribe arginine, glucagon and Synacthen doses.

4. Insert IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer synacthen as a push intravenously followed by a 0.9% saline flush
6. Administer arginine via intravenous infusion over 30 minutes straight after the 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 as outlined in table below.
7. Blood sampling at timepoints as outlined in table below.
8. Administer glucagon subcutaneously (dose as per dosing table above) as soon as+90Min blood sample has been collected.
9. Continue blood sampling at timepoints as outlined in table below.
10. 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.
11. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered			**Dose Administered**						**Time Administered**				
	Baseline		**Minutes post START of arginine infusion**										
Actual time bloods taken		Administer synacthen and arginine						Administer glucagon					
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		150 Min	180 Min	210 Min	240 Min	270 Min
GH	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Glucose	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Cortisol	✓		✓		✓								
ACTH	✓												
Other tests, for example, IGF1, IGFBP3 as per	+/-												

requesting clinician													
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL						

Interpretation:

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH deficient sequelae (for	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an

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.	adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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

	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

*Table adapted from HEDTA

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, initial sub-optimal cortisol response (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

Caution in the interpretation of 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.

Historically, some SST protocols have stipulated that for an adrenal response to be deemed adequate / sufficient, in addition to having a peak cortisol level rise above a certain cut-off threshold, a minimum increment in cortisol level from baseline to peak had to also be achieved. This is however no longer a requirement as individuals with normal adrenal function with a high baseline cortisol level will not achieve this increment.

REFERENCES

See individual protocols

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

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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)

Certain drugs, for example, periactin, interfere with arginine stimulation

People with known allergic tendencies

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

Caution in children with known congenital / acquired heart disease

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

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 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:

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

Anaphylaxis to Tetracosactrin 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:

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

CLONIDINE

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

SYNACTHEN

Formulation	Dose		Route
Tetracosactrin (Synacthen, solution for injection) 250 mcg in 1 mL	0 – 6 months old	62.5 micrograms	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Note:

Clonidine 100 microgram and 150 microgram tablets available on PBS, Australia

Clonidine 25 microgram and 150 microgram tablets available in New Zealand

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Clonidine

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

Hypotension, postural hypotension. Fall in blood pressure by ~10 mmHg about 1 hour after ingestion. Usually resolves by the end of the test but may last several hours. Effect 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.

Synacthen

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

Preparation:

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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 on regular antihypertensive medication, please check with the SMO 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 SST. 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 (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

This test should be performed before 0900 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 SST), then the SST can be performed at any time of day as 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 SMO responsible for the patient about the desired timeframe post-surgery that the SST should be arranged for. Following 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 ~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 a 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, 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 request form.

Sex steroid priming

The evidence and expert opinions regarding sex steroid priming are mixed. The HDET-Paeds Guidelines aim to harmonize paediatric endocrine dynamic testing practice across Australasia.

The HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

Sex steroid priming options for males & females

Formulation	**Dose**	**Duration**
Ethinyloestradiol	40mcg/m2 orally in 2-3 divided doses per day	2 days, reporting next day for GH stimulation testing
Micronized estradiol valerate	Weight ≤ 20kg: 1mg daily orally Weight >20kg: 2mg daily orally	2-3 days, reporting next day for GH stimulation testing

Estradiol side effects: can include moderate and transient breast enlargement. Discontinue if nausea and vomiting occur

Equipment:

Equipment for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, clonidine, Synacthen

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Ideally perform test first thing in the morning following an overnight fast. However, minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh patient, calculate arginine, clonidine and Synacthen doses and take baseline observations.
3. Insert IV cannula and take baseline (pre-stimulation) blood samples.
4. Administer synacthen as a push intravenously followed by a 0.9% saline flush
5. Administer arginine via intravenous infusion over 30 minutes straight after the 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 as outlined in table below.
6. Administer clonidine orally (dose as per dosing table above) as soon as+90Min blood sample has been collected.
7. Consider giving 10 ml/kg IV bolus of 0.9% sodium chloride over 30 minutes following 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.
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 until the test is completed. Water is permitted.

Discharge:

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 abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

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 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL						

Interpretation:

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, for example, arginine, clonidine, glucagon, insulin OR	Current or historical evidence of a diagnostic insulin tolerance test with maximum serum GH < 2.5 mcg/L 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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 GH stimulation tests. In children who are 5 years and older, GH testing with sex steroid priming is required.	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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

	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

*Table adapted from HEDTA

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, initial sub-optimal cortisol response (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

Caution in the interpretation of 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.

Historically, some SST protocols have stipulated that for an adrenal response to be deemed adequate / sufficient, in addition to having a peak cortisol level rise above a certain cut-off threshold, a minimum increment in cortisol level from baseline to peak had to also be achieved. This is however no longer a requirement as individuals with normal adrenal function with a high baseline cortisol level will not achieve this increment.

REFERENCES

See individual protocols

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test: Arginine and Glucagon
GnRH Stimulation Test: Triptorelin (Aus) or Gonadorelin (NZ)

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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 48hours, 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 (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

People with known allergic tendencies

Pregnancy (relative contraindications)

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 stimulation to the pituitary gland and nullification of the 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:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy + ability to site an IV cannula.

Formulation & Dose:

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

GLUCAGON

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

TRIPTORELIN + GONADORELIN

Formulation	**Dose**	**Route**
Australia		
Triptorelin acetate (Decapeptyl 100 micrograms/ml)	100 micrograms/m2 or 2.5 micrograms/kg (max 100 micrograms)	Subcutaneous

Note: DO NOT USE Diphereline (long acting triptorelin)		
New Zealand		
Gonadorelin (HRF, Ayerst)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Glucagon

Transient nausea, flushing, vomiting for 1 – 2 minutes, abdominal pain / cramps, 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.

GnRH (triptorelin, gonadorelin)

Significant adverse reactions have not been encountered. Occasionally subjects may experience nausea and abdominal pain.

Preparation:

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

Ensure the patient has robust intravenous access for 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 request form.

Sex steroid priming

In other circumstances, the HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

HOWEVER: as this combined test includes a 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 for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, glucagon, triptorelin OR gonadorelin

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient and take baseline observations.
3. Work out and prescribe arginine, glucagon and triptorelin/gonadorelin doses.
4. Insert IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer Triptorelin subcutaneously OR Gonadorelin intravenously as a slow push over 1 minute.
6. Administer arginine via intravenous infusion over 30 minutes immediately following administration of gonadorelin OR triptorelin (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 will need to be done immediately (following flush) after completion of the arginine infusion. Blood sampling at timepoints as outlined in table below
7. Administer glucagon as soon as+90Min blood sample has been collected.
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.

10. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered			**Dose Administered**						**Time Administered**						
	Baseline		**Minutes post START of arginine infusion**												
Actual time bloods taken		Administer arginine and triptorelin OR gonadorelin						Administer glucagon							
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	240 Min	270 Min	24 Hr
GH	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
Glucose	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
LH and FSH Triptorelin used	✓		✓		✓				✓		✓				
LH and FSH Gonadorelin used	✓		✓	✓	✓										
Testosterone (males) Estradiol (females)	✓														✓
Other tests e.g. IGF1, IGFBP3, ACTH cortisol as per requesting clinician	+/-														
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL								

*See Notes section below (Timing of post-triptorelin/gonadorelin stimulation blood sampling note)

Interpretation:

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies across different centres throughout the world, and between paediatric and adult practice. GH level cut-off thresholds that are currently in use range from GH < 0.4 mcg/L to GH < 10 mcg/L.

To access funded growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PBS and PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, of which one should be ITT unless contraindicated. Where an

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.	additional test is required, an arginine provocation test can be used with a peak serum GH ≤ 0.4 mcg/L.

GnRH Stimulation Test Interpretation

The most widely accepted cut-off concentration for the LH peak suggestive of HPG axis activation is 5.0 IU/L

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

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/GnRHa used, the sampling timepoints used, and the LH assay used.

If only taking blood samples at baseline and 1-2 timepoint post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, from 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 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 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 isn't required to rule in/rule out a diagnosis of 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 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 when it comes to 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 intravenous injection (0.1mg/m^2 body surface area, maximum dose 0.1mg):

	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)

During infancy, usually between 1 – 6 months of age, there is transient activation of the HPG axis, termed ‘mini-puberty of infancy’. Performing a GnRH stimulation test during 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) or Gonadorelin (NZ)

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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 GH response)

Certain drugs, for example, periactin, interfere with arginine stimulation

People with known allergic tendencies

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

Caution in children with known congenital / acquired heart disease

Pregnancy (relative contraindications)

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 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:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and ability to site an IV cannula.

Anaphylaxis to Tetracosactrin 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:

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

CLONIDINE

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

TRIPTORELIN or GONADORELIN

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)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)

Note:

Clonidine 100 microgram and 150 microgram tablets available on PBS, Australia

Clonidine 25 microgram and 150 microgram tablets available in New Zealand

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Clonidine

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

Hypotension, postural hypotension. Fall in blood pressure by ~10 mmHg about 1 hour after ingestion. Usually resolves by the end of the test but may last several hours. Effect 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.

GnRH (triptorelin, gonadorelin)

Significant adverse reactions have not been encountered. Occasionally subjects may experience nausea and abdominal pain.

Preparation:

Ensure patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

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

If on regular antihypertensive medication, please check with the SMO 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 request form.

Sex steroid priming

In other circumstances, the HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

HOWEVER: as this combined test includes a 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 for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, clonidine, triptorelin OR gonadorelin

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Ideally perform test first thing in the morning following an overnight fast. However, minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh patient and take baseline observations.
3. Calculate and prescribe arginine, clonidine, and triptorelin/gonadorelin doses.
4. Insert IV cannula and take baseline (pre-stimulation) blood samples.
7. Administer Triptorelin subcutaneously OR Gonadorelin intravenously as a slow push over 1 minute.

6. Administer arginine via intravenous infusion over 30 minutes immediately following administration of gonadorelin OR triptorelin (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 will need to be done immediately (following flush) after completion of the arginine infusion. Blood sampling at timepoints as outlined in table below.
7. Blood sampling at timepoints as outlined in table below
8. Administer clonidine orally as soon as +90 min blood sample has been collected.
9. Continue blood sampling at timepoints as outlined in table below.
10. 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.
11. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered				**Dose Administered**							**Time Administered**				
	Baseline		**Minutes post START of arginine infusion**												
Actual time bloods taken		Administer arginine, and triptorelin or gonadorelin						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	✓													
Triptorelin used	✓		✓		✓				✓		✓			

LH and FSH	Gonadorelin used	✓		✓	✓	✓										
Testosterone (males) Estradiol (females)		✓														✓
Other tests e.g. IGF1, IGFBP3, ACTH cortisol as per requesting clinician		+/-														
Sample Tubes / Minimum Blood Volume		SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST 1mL								

*See Notes section below (Timing of post-triptorelin/gonadorelin stimulation blood sampling note)

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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	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

• 1 GHST (pharmacological or physiological) and low plasma IGFBP-3 levels	

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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 GH stimulation tests. In children who are 5 years and older, GH testing with sex steroid priming is required.	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

GnRH Stimulation Test Interpretation

LH peak post-GnRH agonist ≥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.

See Notes section below regarding the use and interpretation of GnRH stimulation test for 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 (may also occur in 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 stage 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 estradiol 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 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/GnRHa used, the sampling timepoints used, and the LH assay used.

If only taking blood samples at baseline and 1-2 timepoint post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, from 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 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 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 for CPP. Other studies report that this isn't required to rule in/rule out a diagnosis of CPP. The 24 hour post-GnRH/GnRHa stimulation testosterone/estradiol 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 when it comes to 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 intravenous injection (0.1mg/m^2 body surface area, maximum dose 0.1mg):

	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)

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

REFERENCES

See individual protocols

COMBINED PROTOCOL
Combined Pituitary Function Test
GH Stimulation Test: Arginine and Glucagon
GnRH Stimulation Test: Triptorelin (Aus) or Gonadorelin (NZ)
Short Synacthen Test: Synacthen (ACTH)

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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 48hours, 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 (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

People with known allergic tendencies

Pregnancy (relative contraindications)

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

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 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:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and ability to site an IV cannula.

Anaphylaxis to Tetracosactrin 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:

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

GLUCAGON

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

TRIPTORELIN or GONADORELIN

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)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)

SYNACTHEN

Formulation	Dose		Route
Tetracosactrin (Synacthen, solution for injection) 250 mcg in 1 mL	0 – 6 months old	62.5 micrograms	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Glucagon

Transient nausea, flushing, vomiting for 1 – 2 minutes, abdominal pain / cramps, 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

GnRH (triptorelin, gonadorelin)

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 patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

Ensure the patient has robust intravenous access for 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 SST. 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 (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

This test should be performed before 0900 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 SST), then the SST can be performed at any time of day as 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 SMO responsible for the patient about the desired timeframe post-surgery that the SST should be arranged for. Following 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 ~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 a 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, 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 request form.

Sex steroid priming

In other circumstances, the HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

HOWEVER: as this combined test includes a 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 for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, glucagon, triptorelin OR gonadorelin, Synacthen

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test.
2. Weigh patient and take baseline observations.
3. Work out and prescribe arginine, glucagon, triptorelin/gonadorelin, and Synacthen doses.
4. Insert IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer synacthen, triptorelin/gonadorelin and arginine one after the other
   - 1st: synacthen intravenously as a push
   - 2nd: triptorelin subcutaneously OR gonadorelin intravenously as a slow push over 1 minute
   - 3rd: arginine via intravenous infusion over 30 minutes

   The time that the arginine infusion STARTS (not finishes) is Time 0. Collection of the 30-minute samples for the GHST, SST and GnRH will need to be done immediately (following flush) after completion of the arginine infusion
6. Blood sampling at timepoints as outlined in table below
7. Administer glucagon subcutaneoulsy as soon as +90 min blood sample has been collected.
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.
10. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered				**Dose Administered**						**Time Administered**						
	Baseline		**Minutes post START of arginine infusion**													
Actual time bloods taken		Administer synacthen, triptorelin/gonadorelin and arginine						Administer glucagon								
Test	-1 Min		30 Min	45 Min	60 Min	75 Min	90 Min		120 Min	150 Min	180 Min	210 Min	24 Min	270 Min	24 Hr
GH	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
Glucose	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
Cortisol	✓		✓		✓										
ACTH	✓														
LH and FSH — Triptorelin used	✓		✓		✓				✓		✓				
LH and FSH — Gonadorelin used	✓		✓	✓	✓										
Testosterone (males) Estradiol (females)	✓														✓
Other tests e.g. IGF1, IGFBP3, ACTH cortisol as per requesting clinician	+/-														
Sample Tubes / Minimum Blood Volume	SST 2 mL		SST 1mL	SST 1mL	SST 1mL	SST 1mL	SST								

							1mL								

*See Notes section below (Timing of post-triptorelin/gonadorelin stimulation blood sampling note)

Growth Hormone Stimulation Test Interpretation

The GH level that is used as the cut-off threshold for diagnosing and treating growth hormone deficiency varies in different centres throughout the world, and between paediatric and adult practice. 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 growth hormone treatment in Australia and New Zealand there are different criteria that must be met, and these are determined by PBS (Australia) or PHARMAC (NZ). 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 PHARMAC.

Australia: Biochemical PBS criteria for biochemical growth hormone deficiency

Children	Adults
Peak serum GH < 3.3 mcg/L (<10 mU/L) in response to • 2 pharmacological GHST, 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 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

New Zealand: Biochemical PHARMAC criteria for biochemical growth hormone deficiency

Children	Adults
GH deficiency causing symptomatic hypoglycaemia, or with other significant GH 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)	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an adequately performed insulin tolerance test or glucagon stimulation test.

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.	Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

GnRH Stimulation Test Interpretation

LH peak post-GnRH agonist ≥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.

See Notes section below regarding the use and interpretation of GnRH stimulation test for 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 (may also occur in 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 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 and different immunoassays. The median cortisol levels at 60 minutes have been reported to be approximately 15% higher than 30 minute levels.

	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

*Table adapted from HEDTA

Cortisol level in neonates

In neonates <6 months, initial sub-optimal cortisol response (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

Caution in the interpretation of 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.

Historically, some SST protocols have stipulated that for an adrenal response to be deemed adequate / sufficient, in addition to having a peak cortisol level rise above a certain cut-off threshold, a minimum increment in cortisol level from baseline to peak had to also be achieved. This is however no longer a requirement as individuals with normal adrenal function with a high baseline cortisol level will not achieve this increment.

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 stage 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 estradiol 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 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/GnRHa used, the sampling timepoints used, and the LH assay used.

If only taking blood samples at baseline and 1-2 timepoint post-GnRH/GnRHa stimulation due to time constraints or because of challenges with collecting multiple blood samples, from 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 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 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 isn't required to rule in/rule out a diagnosis of CPP. The 24-hour post-GnRH/GnRHa stimulation testosterone/estradiol 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 when it comes to 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 intravenous injection (0.1mg/m^2 body surface area, maximum dose 0.1mg):

	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	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)

Median (minimum, maximum)						

During infancy, usually between 1 – 6 months of age, there is transient activation of the HPG axis, termed ‘mini-puberty of infancy’. Performing a GnRH stimulation test during 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) or Gonadorelin (NZ)
Short Synacthen Test: Synacthen (ACTH)

Indications:

When there are multiple pituitary hormone deficiencies suspected. This could include individuals with a CNS tumour, 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.

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

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 GH and cortisol response)

Certain drugs, for example, periactin, interfere with arginine stimulation

People with known allergic tendencies

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

Caution in children with known congenital / acquired heart disease

Known hypersensitivity to ACTH. Other listed contraindications apply to ongoing treatment with Synacthen only. Current treatment with supraphysiological doses of glucocorticoids.

Pregnancy (relative contraindications)

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 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:

Minimal requirement for test to be performed in a centre with laboratory staff familiar with paediatric laboratory testing, including paediatric phlebotomy and ability to site an IV cannula.

Anaphylaxis to Tetracosactrin 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:

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

CLONIDINE

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

TRIPTORELIN or GONADORELIN

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)	100 micrograms Note: same dose for all ages and all sizes	Intravenous (slow push over 1 minute)

SYNACTHEN

Formulation	Dose		Route
Tetracosactrin (Synacthen, solution for injection) 250 mcg in 1 mL	0 – 6 months old	62.5 micrograms	Intravenous
	6 months – 2 years old	125 micrograms	Intravenous
	Over 2 years old	250 micrograms	Intravenous

Note:

Clonidine 100 microgram and 150 microgram tablets available on PBS, Australia

Clonidine 25 microgram and 150 microgram tablets available in New Zealand

Adverse reactions:

Arginine

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

Allergic reactions, anaphylaxis – extremely rare; hypotension requiring intravenous fluid replacement has been rarely observed one hour after the arginine infusion has been given

Elevated potassium 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.

Clonidine

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

Hypotension, postural hypotension. Fall in blood pressure by ~10 mmHg about 1 hour after ingestion. Usually resolves by the end of the test but may last several hours. Effect 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.

GnRH (triptorelin, gonadorelin)

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 patient is euthyroid and has normal TFTs prior to commencing test.

Ensure patient has normal electrolytes prior to commencing test.

Overnight fast. 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.

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

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

Ensure the patient has robust intravenous access for 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 SST. 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 (child must be well) under medical supervision to avoid false positives. Check with laboratory for cross-reactivity/interferences (some exogenous glucocorticoids will cross-react with the cortisol assay).

This test should be performed before 0900 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 SST), then the SST can be performed at any time of day as 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 SMO responsible for the patient about the desired timeframe post-surgery that the SST should be arranged for. Following 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 ~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 a 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, 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 request form.

Sex steroid priming

In other circumstances, the HDET-Paeds working group endorse the recommendation to use sex steroid priming in all children aged 8 years and older who are pre-pubertal (Tanner stage < 2) and planning to undergo a GH stimulation test.

HOWEVER: as this combined test includes a 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 for IV cannulation and blood collection

- IV cannula, 2ml and 5 ml syringes, 0.9% saline for IV cannula flushes, blood tubes etc

The stimulants – arginine, clonidine, triptorelin OR gonadorelin, Synacthen

Observations:

Temperature, BP, HR, RR at baseline and then every 15 minutes throughout the test

Method:

1. Ensure the appropriate steps from the Preparation section have been taken prior to proceeding with the test. Ideally perform test first thing in the morning following an overnight fast. However, minimum fasting time of only 2 hours required, and this shorter fasting time should be applied in infants and young children.
2. Weigh patient and take baseline observations.
3. Calculate and prescribe arginine, clonidine, triptorelin/gonadorelin, and Synacthen doses.
4. Insert IV cannula and take baseline (pre-stimulation) blood samples.
5. Administer synacthen, triptorelin/gonadorelin and arginine one after the other
   - 1st: synacthen intravenously as a push
   - 2nd: triptorelin subcutaneously OR gonadorelin intravenously as a slow push over 1 minute
   - 3rd: arginine via intravenous infusion over 30 minutes

   The time that the arginine infusion STARTS (not finishes) is Time 0. Allow time to give a 10 – 15 ml flush with 0.9% saline prior to taking the 30 minute blood sample.

6. Blood sampling at timepoints as outlined in table below
7. Administer clonidine orally as soon as +90 min blood sample has been collected.
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.
10. No food until the test is completed. Water is permitted.

Discharge:

Child must have been fed and have normal observations and blood glucose level. If abnormal, repeat as required. Review by medical personnel prior to discharge.

Sample collection:

Drug Administered			**Dose Administered**						**Time Administered**					
	Baseline		**Minutes post START of arginine infusion**											
Actual time bloods taken		Administer synacthen, triptorelin/gonadorelin 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 + FSH (Triptorelin)	✓		✓		✓				✓		✓			
LH and FSH (Gonadorelin)	✓		✓	✓	✓									
Testosterone (males) Estradiol (females)	✓													✓
Other tests e.g. IGF1, IGFBP3, ACTH cortisol as per requesting clinician	+/-													

Sample Tubes / Minimum Blood Volume														

*See Notes section below (Timing of post-triptorelin/gonadorelin stimulation blood sampling note)

*24 hour testosterone/estradiol sample is used in the assessment of delayed puberty; check with SMO responsible for patient if it is needed

Growth Hormone Stimulation Test Interpretation

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

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GH deficiency causing symptomatic hypoglycaemia, or with other significant GH deficient sequelae (for	For adults and adolescents, severe GH deficiency is defined as peak serum GH level ≤ 3 mcg/L during an

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.	adequately performed insulin tolerance test or glucagon stimulation test. Patients with 1 or more additional anterior pituitary hormone deficiencies and a known structural pituitary lesion only require one test. Patients with isolated GHD require 2 GHST, 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.

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

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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 when it comes to 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 intravenous injection (0.1mg/m^2 body surface area, maximum dose 0.1mg):

	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)	26.56	20.51	20.14	12.15	17.22	11.53

Median (minimum, maximum)	(22.82-40.39)	(16.62-29.43)	(9.11-36.15)	(7.94-19.00)	(10.40-20.69)	(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)

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

REFERENCES

See individual protocols