Harmonisation of Endocrine Dynamic Testing -Adult (HEDTA)

This manual is a joint initiative from ESA/AACB/RCPA and is freely available as a resource for Endocrinologists and Biochemists.

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Available at:

https://www.endocrinesociety.org.au/guidelines.asp

https://www.aacb.asn.au/documents/item/5429

The information provided is a guide only and needs to be verified by individual clinicians and laboratories prior to use. Modifications might be required according to local procedures (e.g., patient consent, sample type, name of test set, collection, interpretation and reporting procedures). Queries can be directed to the chair of the HEDTA working party. A separate paediatric endocrine dynamic testing protocol is in progress with the HDET-P working party.

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Endocrine Analyte Reporting Unit and Sample Tube

The following table is provided as a guide, check with local laboratory for sample type.

Test	Preferred unit	Conventional unit	Conversion to Conventional unit	Collection tube	Collection comment
5-HIAA	umol/day			24 hr urine acid bottle	See Appendix.
ACTH	pmol/L	pg/mL ng/L	4.54	EDTA	Collect on ice
Aldosterone	pmol/L	ng/dL	0.036	EDTA/ serum	State erect (seated for 10 mins) or supine (for 30 mins) on request slip
Aldosterone-Urine	pmol/L	ng/dL	0.036	plain container	Urine 24 h Keep the container refrigerated during the collection period
AVP / ADH	pmol/L	pg/mL	0.923	2 x 4 ml EDTA	Collect on ice
Androstenedione	nmol/L	ng/dL	28.65	Serum	
βHCG	IU/L	mIU/mL	1	Li heparin/ serum	
C-peptide	nmol/L	ng/mL	0.331	Li heparin/ serum	Fasting
Calcitonin	pmol/L	pg/mL	0.292	Li heparin or serum tube NOT EDTA.	Fasting specimen is preferred. Collect on ice.
Chromogranin A	ug/L			Serum	Fasting specimen preferred. Collect on ice.
Copeptin	pmol/L			Li heparin	
Cortisol	nmol/L	ug/dL	27.588	Li heparin/ serum	Morning 8 – 9 am sample
Cortisol - Saliva	nmol/L	ug/dL	27.588	Salivette	11 pm to midnight collection. Nil by mouth/ no teeth brushing 30 mins prior.
DHEAS	umol/L	ug/dL	0.027	Serum	
Estradiol	pmol/L	pg/mL	0.272	Serum	

Free T3	pmol/L	pg/dL	64.9	Li heparin/ serum	
Free T4	pmol/L	ng/dL	0.0775	Li heparin/ serum	
FSH	IU/L	mIU/mL	1	Li heparin/ serum	
Gastrin	pmol/L	pg/mL	0.481	Serum	Fasting, collect on ice. Proton pump inhibitors elevate result.
Glucagon	ng/L	pg/mL	1	EDTA & trasylol containing GLASS tube *(Use 6 mg Na EDTA + 2,500 KIU trasylol per 5 ml blood)*	Fasting. Collect on ice
Glucose	mmol/L	mg/dL	18	Fluoride oxalate/ Na F-EDTA-citrate	Li heparin/ serum if rapid transport to laboratory
GH	ug/L	mU/L	3	Li heparin or Serum tube	
Insulin	pmol/L	mU/L	0.144	Serum	Fasting. Collect on ice
Insulin Ab	unit			Serum	
IGF-1	nmol/L	ng/mL	0.131	Serum or Li heparin	
IGF BP3	nmol/L			Serum	Collect on ice
LH	IU/L			Li heparin/ serum	
17-OHP	nmol/L			Serum	
Metanephrines (plasma)	pmol/L			Li heparin	Fasting, collect on ice, supine for 30 mins. 3-methoxytyramine might need to be specified on request if required
Osteocalcin	ug/L			Serum, Li-heparin or K3 EDTA	
Pancreatic polypeptide	pmol/L			Serum	Fasting, morning sample

PTH	pmol/L	pg/mL ng/L	9.4	Serum or EDTA	Serum tubes to send immediately
PTH-rp	pmol/L			EDTA with Aprotinin additive	Collect on ice
Progesterone	nmol/L	ng/dL	31.44	Li heparin/ serum	
Prolactin	mIU/L	ug/L	21.3	Li heparin/ serum	EDTA plasma possible for most assays for IPSS
Renin mass	mIU/L			EDTA	Do NOT collect on ice. State erect or supine on request slip as per aldosterone
SHBG	nmol/L	ug/mL	8.896	serum or Li heparin	
Steroid profile-Urine				plain container	24-hr urine
Sulphonylurea Screen				Serum/ plasma	Collect during hypoglycemia, only detect ingestion within 24 hrs
VIP	pmol/L	pg/mL	3.38	EDTA & trasylol containing GLASS tube *(Use 6 mg Na EDTA + 2,500 KIU trasylol per 5 ml blood)*	Fasting, collect on ice
Testosterone	nmol/L	ng/dL	28.8	Li heparin/ serum	Fasting, morning 8-9 am sample
TSH	mIU/L			Li heparin/ serum	

1 Adrenal

1.1 SHORT SYNACTHEN TEST

RATIONALE:

The cortisol response to Synacthen (Tetracosactide) stimulation will be low or absent due to primary adrenal pathology (e.g. Addison's disease, bilateral adrenal infiltration) or adrenal atrophy secondary to severe ACTH deficiency of at least 4 weeks' duration. (1) This test does not assess adequacy of ACTH/ CRH response to stress if pathology was of short duration. This is assessed by the ITT or overnight metyrapone test.

Also used for diagnosis of non-classical 21-hydroxylase deficiency, if a morning, screening follicular phase 17 OH progesterone is > 6 nmol/L (lower level for mass spectroscopy assay). For other causes of congenital adrenal hyperplasia, contact laboratory for required tests.

PREPARATION AND PROCEDURE:

1) Withhold any steroid treatment for 24 hours prior to the test (patients treated with dexamethasone require at least 48 hours of steroid withdrawal) if appropriate.

2) Baseline blood is collected for cortisol and ACTH. Procedure should be performed between 8 - 9:30am when cortisol peak is present.

3) IM or IV Synacthen (Tetracosactide) 250 ug

4) Blood for cortisol collected at 30 and 60 minutes

Time	Procedure/ Test	Comment
Baseline	ACTH, cortisol	Also 17-OH progesterone if CAH queried
0 minute	IV or IM Synacthen (Tetracosactide) 250 ug	
30 minutes	cortisol	Also 17-OH progesterone if CAH queried
60 minutes	cortisol	Also 17-OH progesterone if CAH queried

INTERPRETATION:

- Normal SST requires a cortisol from at least one time point to exceed the minimum peak cortisol cut-off specified for that assay. The concentration of peak cortisol cut-off is assay dependent, and for female, OCP raises total cortisol level due to rise in CBG. (1)
- The use of historical peak cortisol of 550 nmol/L in newer cortisol-specific assays may result in false positive results. (2) Previous requirement for a minimum cortisol increment from baseline (e.g. 250 nmol/L) is also redundant as normal individuals with high baseline cortisol will not achieve this increment.
  Laboratories need to determine their own individual cut-off. The table below describes the minimum cortisol level achieved post synacthen (Tetracosactide) at 30 minutes for different immunoassays. (3) The 60 minutes cortisol level was reported to be around 15% higher than the 30 minutes level. (4)

- **The suggested assay specific cut off is in Table 2, after taking into account the imprecision of the assay**

Minimum peak cortisol cut-off (2.5th centile) for healthy subjects 30 mins and 60 mins post IV Synacthen (2):

Cortisol Assay (nmol/L)	Male		Female		Female (OCP)	
	30 mins	60 mins	30 mins	60 mins	30 mins	60 mins
GC-MS	420	483	420	483	640	736
Siemen Centaur	450	518	450	518	620	713
Abbott Architect	430	495	420	483	580	667
Roche E170	420	483	420	483	640	736
Beckman Access	420	483	420	483	640	736
Siemen Immulite	470	541	480	552	690	794

60 mins value is based on the average rise of 15% from the 30 mins cortisol concentration. (4)

Suggested minimum peak cortisol cut-off for healthy subjects at either 30 mins and 60 mins timepoints with indeterminate zone from uncertainty of measurement

Cortisol Assay (nmol/L)	Male and Female not on OCP		Female (OCP)	
	Cut-off	Borderline Zone	**Cut-off**	Borderline Zone
GC-MS	**490**	440 - 530	**740**	660 - 810
Siemen Centaur	**520**	470 - 570	**720**	640 - 780
Abbott Architect	**500**	450 - 550	**670**	600 - 730
Roche E170	**490**	440 - 530	**740**	660 - 810
Beckman Access	**490**	440 - 530	**740**	660 - 810
Siemen Immulite	**550**	490 - 600	**800**	720 - 870

INTERPRETATION for ADRENAL CORTISOL DEFICIENCY: (5)

Test outcome	**Suggested comment:**
Normal SST	The short Synacthen test was normal, stimulated cortisol level exceeded (the minimum peak cortisol cut-off specified for that assay). This does not exclude acute secondary hypocortisolism.
Abnormal SST	The short Synacthen test was abnormal, stimulated cortisol levels were below (the minimum peak cortisol cut-off specified for that assay).

INTERPRETATION for 21 HYDROXYLASE DEFICIENCY CAH: (6)

Test outcome	**Suggested comment:**
Stimulated 17 OH progesterone > 43 nmol/L	An exaggerated rise of 17 OH progesterone post ACTH stimulation (>43 nmol/L) is seen in 21 hydroxylase deficiency CAH, and some patients with adrenal adenomas.

Stimulated 17 OH progesterone 30 - 43 nmol/L	A moderate rise of 17 OH progesterone post ACTH stimulation (30-43 nmol/L) constitutes a grey zone whereby genetic tests might be required to confirm or exclude 21 hydroxylase deficiency CAH.
Stimulated 17 OH progesterone < 30 nmol/L	A mild rise of 17 OH progesterone post ACTH stimulation (< 30 nmol/L) is a normal physiological response and not consistent with 21 hydroxylase deficiency CAH.

The cut-offs for 17 OH progesterone in the table are based on radioimmunoassay, LCMS cut-off for 17 OH progesterone is lower at 9 nmol/L. (7)

NOTES:

- Nausea, palpitation, hot flushes or allergic reaction can rarely occur with synacthen (Tetracosactide).
- Although IV administration is preferred, IM administration is also valid, however cortisol at 30 minutes is more variable. (8)
- SST result is difficult to interpret in critically ill patients due to difficulties in interpreting total cortisol results from immunoassays. (9)

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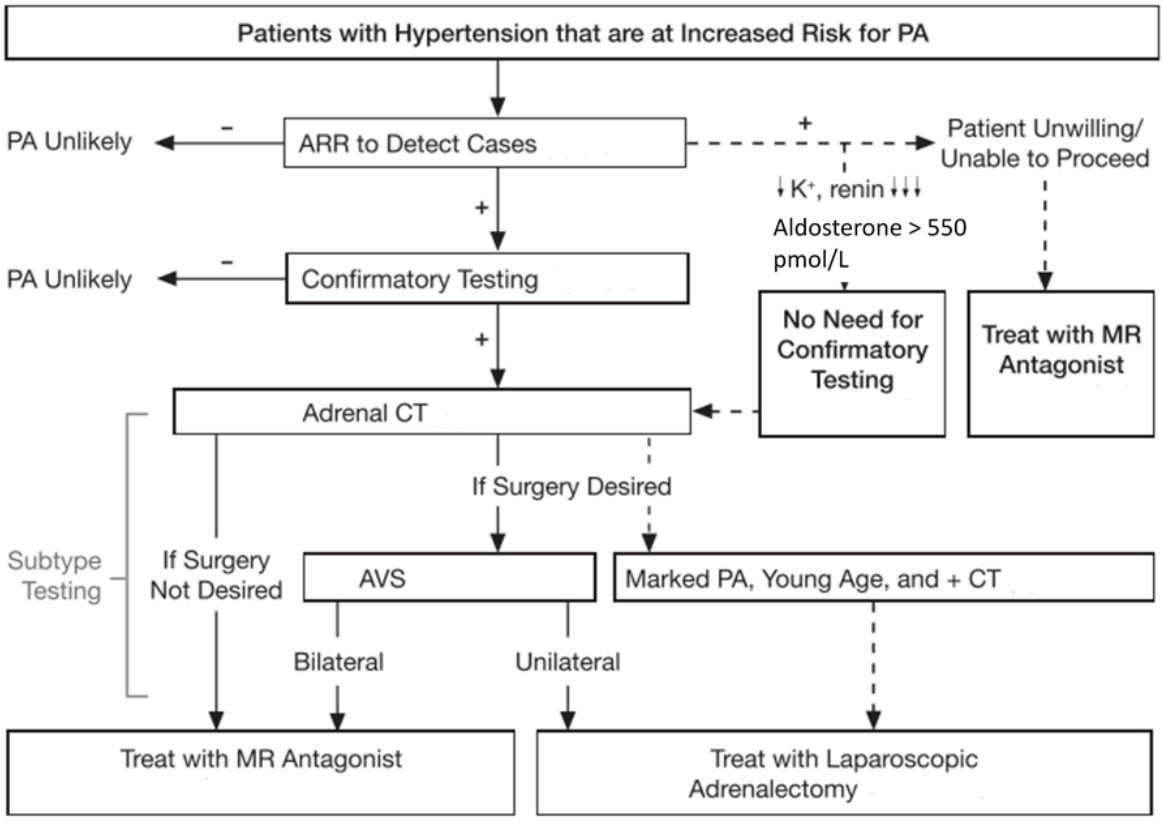
1.2 PRIMARY ALDOSTERONISM INVESTIGATION

PATIENT PREPARATION



Algorithm for the detection, confirmation, subtype testing, and treatment of Primary hyperaldosteronism (PA). Adapted from Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. (1)

- Interfering drugs which can affect renin, aldosterone or both should be stopped for at least
  - 4 weeks: Spironolactone, eplerenone, amiloride, and triamterene, potassium-wasting diuretics, licorice.
  - 2 weeks: Angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, and dihydropyridine calcium channel antagonists, clonidine, methydopa, beta-blockers.
- Drugs which do not affect renin, aldosterone for blood pressure control includes: verapamil slow-release, prazosin, hydralazine, moxonidine.
- Hypokalemia needs to be corrected.
- Aldosterone:renin ratio (ARR) is the preferred screening test. Preferably two elevated values should be obtained prior to confirmation testing. The ARR test is most sensitive when samples are collected in the morning after patients have been out of bed for at least 2 hours, usually after they have been seated for 5–15 minutes.

- There is no gold standard for confirmation testing. Of the 4 testing procedures available, captopril challenge test can have false negative equivocal results and therefore not mentioned in this document. (1) Oral sodium loading test requires sensitive and specific urinary aldosterone measurement (LC-MSMS) in patients without renal impairment.
- In florid Primary aldosteronism (hypokalemia, suppressed renin, elevated aldosterone > 550 pmol/L), confirmation tests might not be required.

1.3 SALINE SUPPRESSION TEST

RATIONALE:

Confirmation test when aldosterone:renin ratio (ARR) elevated. Test should not be performed in patients with uncontrolled hypertension, hypokalaemia, arrhythmias, severe CCF or renal failure. Saline infusion acts as a salt and fluid load, suppresses aldosterone production in normal subjects but not in subjects with primary aldosteronism.

PROCEDURE:

Ensure normokalaemia (target K+ ≥ 4 mmol/L). Check K+ prior to procedure date to adjust potassium supplement dose.

1) Seated procedure

Time	Procedure	Comment
-30 minutes	Patient seated for 30mins prior to and during infusion	
Baseline	Renin, aldosterone, U+Es, cortisol, Venous Blood Gas for rapid K	Patient remains seated throughout procedure
0 minute	2L 0.9% Normal Saline IV over 4hours	
4 hours	Renin, aldosterone, U+Es, cortisol	

2) Recumbent procedure

Time	Procedure	Comment
-60 minutes	Patient recumbent for 60mins prior to and during infusion	
Baseline	Renin, aldosterone, U+Es, cortisol, Venous Blood Gas for rapid K	Patient remains recumbent throughout procedure
0 minute	2L 0.9% Normal Saline IV over 4hours	
4 hours	Renin, aldosterone, U+Es, cortisol	

INTERPRETATION:

Primary aldosteronism is confirmed if all of the conditions below are met:

1) K+ level ≥ 4.0 mmol/L.

2) Post infusion cortisol is less than basal cortisol to exclude confounding ACTH effect

3) Post infusion aldosterone cut off:

A) Seated procedure (6) (7)

- Liaison immunoassay
  - ≤ 170 pmol/L = PA unlikely (sensitivity 95%. specificity 80%)
  - 171-220pmol/L = borderline zone
  - > 220 pmol/L = PA likely (sensitivity 86%. specificity 87%) #
- LCMS < 160 pmol/L = PA unlikely
- IDS (iSYS) immunoassay
  - ≤ 140 pmol/L = PA unlikely

B) Recumbent procedure (cut-offs based on immunoassays)

- < 140 pmol/L = PA unlikely
- 140 – 280 pmol/L = borderline
- >280 pmol/L = PA likely

NOTES:

- Fluid status check should take place during infusion, particularly for those prone to fluid overload.
- Seated normal saline suppression test was found to have higher sensitivity compared to supine normal saline suppression and has good agreement with the more cumbersome fludrocortisone suppression test. (2) (5)
- Aldosterone cut offs are lower if measured using LCMS compared to immunoassays.
- # 220 pmol/L rounded from 217 pmol/L in reference (6)

1.4 FLUDROCORTISONE SUPPRESSION TEST

RATIONALE:

Confirmation test when aldosterone:renin ratio (ARR) elevated. Test should not be performed in patients with uncontrolled hypertension, hypokalaemia, arrhythmias, severe CCF or renal failure. Fludrocortisone, a potent mineralocorticoid, suppresses aldosterone production in normal subjects but not in subjects with primary aldosteronism.

PROCEDURE:

1) Most patients require admission to monitor BP and K+ status
2) Ensure normokalaemia throughout the procedure with oral potassium supplements (target K+ at least 4 mmol/L)
3) Salt loading is required, e.g., a liberalized dietary sodium intake, supplemented by Slow-Na 10 mmol three tablets TDS, target urine Na excretion > 3 mmol/kg/day
4) Collect 24 hr urine for aldosterone, sodium, potassium and creatinine levels 1 day prior to fludrocortisone administration and again on the last 24h
5) Give Fludrocortisone 0.1 mg every 6 hours for 4 days (0400,1000,1600,2200)
6) Daily blood test for renin, aldosterone, U+Es, cortisol. Extra blood test might be required for Slow K dosing
7) Blood test at 0700 and 1000 on Day 5 are required for interpretation

Time	**Procedure**	**Comment**
- 1 Day	24 hr urine aldosterone, sodium, potassium and creatinine	Ensure normokalaemia (target K+ ≥ 4 mmol/L) and salt loading for the 4 days of test. Check K+ to adjust potassium supplement dose
Day 1: 0700	Recumbent: Renin, aldosterone, U+Es, cortisol	
Day 1: 1000	Upright: Renin, aldosterone, U+Es, cortisol	Fludrocortisone 0.1 mg every 6 hours (1000,1600,2200)
Day 1: 1600	Check K+	Optional to ensure K remains at target
Day 2: 0700	Recumbent: Renin, aldosterone, U+Es, cortisol	Fludrocortisone 0.1 mg every 6 hours (0400,1000,1600,2200)
Day 2: 1000	Upright: Renin, aldosterone, U+Es, cortisol	
Day 2: 1600	Check K+	Optional to ensure K remains at target
Day 3: 0700	Recumbent: Renin, aldosterone, U+Es, cortisol	Fludrocortisone 0.1 mg every 6 hours (0400,1000,1600,2200)
Day 3: 1000	Upright: Renin, aldosterone, U+Es, cortisol	
Day 3: 1600	Check K+	Optional to ensure K remains at target
Day 4: 0700	Recumbent: Renin, aldosterone, U+Es, cortisol	Fludrocortisone 0.1 mg every 6 hours (0400,1000,1600,2200)
Day 4: 1000	Upright: Renin, aldosterone, U+Es, cortisol 24 hr urine aldosterone, sodium, potassium and creatinine	
Day 4: 1600	Check K+	Optional to ensure K remains at target

Day 5: 0700	Recumbent: Renin, aldosterone, U+Es, cortisol	Last dose of fludrocortisone Day 5 at 0400.
Day 5: 1000	Upright: Renin, aldosterone, U+Es, cortisol	

INTERPRETATION:

Primary aldosteronism is confirmed if all of the conditions below are met:

1) upright aldosterone levels on Day 5 (4 days of fludrocortisone) are > 170 pmol/L (1)
2) upright renin on Day 5 is suppressed.
3) K+ level normal (at least 4.0 mmol/L) on Day 5
4) Plasma cortisol on Day 5 does not increase significantly from 0700h to 1000h (increase may indicate ACTH stimulation of aldosterone production that may have prevented suppression).

NOTES:

- Blood pressure and fluid status check should take place during fludrocortisone and salt loading.
- Aldosterone cut off is lower (down to 130 pmol/L) if measured using LCMS rather than immunoassay, consult laboratory for cut-off.

1.5 ORAL SODIUM LOADING TEST

RATIONALE:

Confirmation test when aldosterone:renin ratio (ARR) elevated. Test should not be performed in patients with uncontrolled hypertension, hypokalaemia, arrhythmias, severe CCF or renal insufficiency. Oral sodium suppresses aldosterone production in normal subjects but not in subjects with primary aldosteronism.

PROCEDURE:

1) Ensure normokalaemia (target K+ ≥ 4 mmol/L). Check K+ prior to procedure date to adjust potassium supplement dose.
2) Oral sodium 200 mmol or 6 g daily
3) 24 hr urine collection for aldosterone and Na starting on Day 3
4) Urine aldosterone needs analysis on a specific assay

Time	Procedure	Comment
Day 1	Oral sodium 200 mmol or 6 g daily	
Day 2	Oral sodium 200 mmol or 6 g daily	
Day 3	Oral sodium 200 mmol or 6 g daily	Start 24 hr urine (aldosterone and Na) collection 8am after discarding first void urine
Day 4		Complete 24 hr urine (aldosterone and Na) collection 8am

INTERPRETATION:

Elevated 24 hr urine aldosterone > 33 nmol/day by LCMS method makes primary hyperaldosteronism likely, providing 24 hr urine Na is elevated (urine Na excretion >3 mmol/kg/day). Consult laboratory for local cut-off.

NOTES:

Non-specific aldosterone methods may blunt diagnostic accuracy due to cross-reactivity with other metabolites in urine.

1.6 ADRENAL VENOUS SAMPLING

RATIONALE:

In patients with confirmed primary aldosteronism (PA) who are surgical candidates, adrenal venous sampling (AVS) is the gold standard in lateralisation of the source of aldosterone excess and differentiates between unilateral adrenal adenoma from bilateral adrenal hyperplasia. All patients should have adrenal CT prior to AVS to exclude large (> 4 cm) adrenal masses.
CT and MRI can misdiagnose the cause of PA. Therefore, AVS is still required for lateralisation with the exception of younger patients < 35 years with spontaneous hypokalaemia, marked aldosterone excess, and unilateral adrenal cortical adenoma on CT who might be able to proceed directly to unilateral adrenalectomy. (1)
AVS should be performed by experienced interventional radiologist. The use of ACTH stimulation is used to improve successful cannulation rate by increasing the adrenal to periphery gradient and to minimise stress induced fluctuation in sequential adrenal vein sampling. Point of care cortisol kit during AVS also increased cannulation rates. (4)

PROCEDURE:

1) Book AVS with experienced interventional radiologist. Notify laboratory of test.
2) AVS can be
    a) Unstimulated: only baseline AVS samples collected in early morning after overnight recumbency.
    b) Stimulated with Synacthen (Tetracosactide): baseline and post ACTH AVS samples collected. Synacthen protocols include:
        I. Bolus 250 ug Synacthen 15 mins before stimulated AVS collection
        II. Continuous 50 ug/hr Synacthen (250ug in 500ml N saline, 100ml per hr) 30 mins before stimulated AVS collection and continued until AVS completion
        III. Bolus 250 ug Synacthen followed by 50ug/hr Synacthen 15- 30 mins before stimulated AVS collection and continued until AVS completion
3) Tubes should be pre-labelled with site, baseline/ stimulated samples and collected in duplicate at each site for each timepoint.

Baseline AVS collection

Time	Procedure	Comment
Document time on tube	Common femoral vein is punctured and a 5 French sheath inserted. *Peripheral* cubital fossa blood taken for aldosterone and cortisol	
Document time on tube	Selective catheterization of *left adrenal vein* and take blood for aldosterone and cortisol	
Document time on tube	Selective catheterization of *right adrenal vein* and take blood for aldosterone and cortisol	Right adrenal vein is more difficult to cannulate.

Stimulated AVS collection (post Synacthen stimulation)

Time	Procedure	Comment
	Synacthen stimulation	See procedure above
Document time on tube	*Peripheral* cubital fossa blood taken for aldosterone and cortisol	Procedure starts 15 to 30 mins post Synacthen administration depending on protocol used
Document time on tube	Selective catheterization of *right adrenal vein* and take blood for aldosterone and cortisol	Right adrenal vein is more difficult to cannulate
Document time on tube	Selective catheterization of *left adrenal vein* and take blood for aldosterone and cortisol	

INTERPRETATION:

Lateralisation of aldosterone excess is present if all of the following are present:

1) Both adrenal veins were successfully cannulated (adrenal vein cortisol: peripheral cortisol ≥ 2 at baseline, ≥ 3 post ACTH).

2) ACTH stimulated AVS:

Aldosterone:cortisol ratio (ACR) between the two adrenals is

> 4 = lateralisation

3 – 4 = borderline

< 3 = no evidence of lateralisation

3) Unstimulated AVS:

Aldosterone:cortisol ratio (ACR) between the two adrenals is

> 2 = lateralisation

4) The unaffected adrenal gland should have an ACR < periphery ACR to indicate suppression by the contralateral unaffected side.

Lack of lateralisation can occur in a) bilateral aldosterone producing adenoma, b) bilateral adrenal hyperplasia, c) Glucocorticoid Remediable Aldosteronism.

NOTES:

- Sample worksheet on next page
- Adrenal haemorrhage can occur in up to 2.5% of AVS procedures
- In patients < 20 years old with confirmed PA or in those who have a family history of PA or strokes < 40 years old, genetic testing for FH-I (Glucocorticoid Remediable Aldosteronism, hybrid CYP11B1/CYP11B2 mutation) should be considered. (1)
- In patients with confirmed PA presenting in childhood, germline mutations in KCNJ5 causing FH-III should be considered.
- Glucocorticoid Remediable Aldosteronism mutation testing replaces indirect test such as dexamethasone suppression test.

	Pre-Synacthen		Post-Synacthen		
Right AV	Time:		Time:		
Aldosterone					pmol/L
Cortisol					nmol/L
Aldo:Cort Ratio ^	**#DIV/0!**	######	**#VALUE!**	**#VALUE!**	
Left AV	Time:		Time:		
Aldosterone					pmol/L
Cortisol					nmol/L
Aldo:Cort Ratio ^	**#VALUE!**	######	**#VALUE!**	**#VALUE!**	
Periphery	Time:		Time:		
Aldosterone					pmol/L
Cortisol					nmol/L
Aldo:Cort Ratio ^	**#VALUE!**	######	**#VALUE!**	**#VALUE!**	
R A:C to **L** A:C #	**#DIV/0!**	######	**#VALUE!**	**#VALUE!**	
L A:C to **R** A:C #	**#VALUE!**	######	**#VALUE!**	**#VALUE!**	
R:P C:C*	#VALUE!	#######	#VALUE!	#VALUE!	
L:P C:C *	#VALUE!	#######	#VALUE!	#VALUE!	

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2 Cushing Overview

Screening investigations: (exclude exogenous glucocorticoids) (1)

1.1 Overnight 1 mg dexamethasone suppression test
1.2 Late night or 11pm salivary cortisol
1.3 24-hour UFC

Consider additional screening tests (if above results are equivocal or discrepant or to exclude pseudo-Cushing's)

1.4 2-day low dose oral dexamethasone suppression
1.5 Dexamethasone-CRH test
1.6 IV 4 mg dexamethasone suppression test

Cushing's syndrome confirmed – measure plasma ACTH

- Suppressed ACTH (< 10ng/L or 2 pmol/L) – adrenal imaging studies for ACTH independent Cushing's
- Borderline ACTH (10-20ng/L or 2-4pmol/L) – consider peripheral CRH test
- Normal or elevated ACTH (> 20ng/L or 4 pmol/L) – proceed to further differential diagnostic tests for ACTH dependent Cushing's

Differential diagnosis of ACTH-dependent Cushing's syndrome

Differentiate between pituitary and ectopic source of ACTH

A. High Dose 8 mg Dexamethasone Suppression Test (not required if IV 4mg dexamethasone test already performed)
B. Peripheral CRH Test
C. Bilateral Inferior Petrosal Sinus Sampling

2.1 OVERNIGHT DEXAMETHASONE SUPPRESSION TEST (1 mg DST)

RATIONALE:

Almost all sources of inappropriate ACTH or cortisol hypersecretion will not be inhibited by 1 mg dexamethasone, therefore this is an excellent screening test and is the recommended screening test for adrenal incidentaloma.

PREPARATION & PROCEDURE:

1. Exclude exogenous glucocorticoid use and medications that may induce metabolism of dexamethasone. Ensure female patients are not on oral oestrogen therapy (stop oral contraceptive pill for at least 4 weeks since oestrogens increase cortisol binding globulin and total cortisol) and advice barrier contraception as required. Transdermal oestrogen does not affect cortisol binding globulin and does not need to be ceased. (15)
2. Dexamethasone 1 mg (2x 0.5 mg tablets) to be given to patient (prescription of dexamethasone may be required)
3. Patient instructed to take dexamethasone at 11 pm.
4. Patient to present to laboratory collection centre for blood test between 8-9am for serum cortisol and ACTH.

Time	Procedure	Comment
11pm	Take 1 mg dexamethasone tablets with water	Ensure strict compliance.
8-9am the next morning	Cortisol	Measure dexamethasone level if querying compliance/ absorption/ metabolism issue.

INTERPRETATION :

Test outcome	Suggested comment:
Normal 1mg DST	A morning cortisol of < 50 nmol/L after 1 mg dexamethasone administered overnight indicates normal suppression of the hypothalamic pituitary adrenal axis.
Abnormal 1mg DST	A morning cortisol ≥ 50 nmol/L after 1 mg dexamethasone administered overnight suggests hypercortisolism.

False positive responses – non-compliance, malabsorption, drugs inducing the hepatic metabolism of dexamethasone (phenytoin, carbamazepine, phenobarbital, rifampicin), drugs elevating CBG (oestrogens), chronic kidney disease, acute illness and pseudo-Cushing's (depression, alcohol abuse, eating disorders).

False negative responses – nephrotic syndrome (↓CBG), renal dialysis, chronic liver disease (reduced metabolism and clearance of dexamethasone).

Sensitivity 98-100%, Specificity 85%

NOTES:

- Hospitalised inpatients not infrequently have an abnormal 1 mg dexamethasone suppression test response, if possible, investigations should be delayed until acute illness has subsided.
- To date, assay specific cut-offs for 1 mg DST are not readily available.
- Concurrent quantification of dexamethasone may reduce false-positive rate and improves specificity, this should be considered in patients with abnormal DST (13).

2.2 LATE NIGHT SALIVARY CORTISOL

RATIONALE:

A loss of diurnal variation is seen in patients with Cushing, whereas this is maintained in pseudo-Cushing syndrome. Salivary cortisol is an ultra-filtrate of plasma and therefore reflects free cortisol and is not affected by protein binding. This test is useful for patients with suspected cyclical Cushing due to ease of repetitive testing.

PREPARATION & PROCEDURE:

1. Patient to collect Salivettes from clinic or laboratory collection centre.
2. Do not use steroid inhaler, relax and avoid vigorous activities for 1 hour prior to saliva collection.
3. Do not eat/ drink/ brush or floss teeth for 30 minutes prior to saliva collection.
4. Ensure hands are clean (avoid topical glucocorticoid cream/lotion contamination) and there is no bleeding inside the mouth just prior to collection.
5. Patient should chew on Salivette for 2 minutes (or until saturated with saliva) between 11pm to midnight.
6. Minimum of two separate samples should be collected on different nights.
7. Salivary cortisol is stable at room temperature. Patient should drop or post Salivettes back to laboratory collection centre.

Time	Procedure	Comment
11pm	Chew on salivette provided for 1-2 minutes until saturated.	Collect prior to brushing teeth and nil by mouth for at least 30 mins prior to collection.

INTERPRETATION :

Normal response – Consult reference intervals provided by the laboratory.

False positive responses – smokers and especially patients who chew tobacco, contamination of salivettes with corticosteroid, bleeding of the gum, shift workers.

False negative responses - non-compliance with collection procedure (drinking water dilutes the sample), cyclical Cushing's (off phase).

NOTES:

An excellent test to use in the investigation of cyclical Cushing's syndrome where initial screening tests are negative. Repeat frequently over expected cycle e.g. weekly for 1-2 months as required.

2.3 24 HOUR URINE FREE CORTISOL (UFC)

RATIONALE:

Inappropriate ACTH or cortisol hypersecretion raises urinary excretion of free cortisol. This is not affected by protein binding, but is affected by renal function. This test is useful for patients with suspected cyclical Cushing.

PREPARATION & PROCEDURE:

1. Patient to collect urinary bottle (no preservative) from the collection centre.
2. Ensure complete 24-hour urine collection (laboratory will provide instruction).
3. Avoid excessive water drinking (>3L daily) and avoid glucocorticoid containing preparations.
4. Suggest 2 x UFC on two separate occasions.

Time	Procedure	Comment
Day 1	Discard first morning void in toilet, patient record starting time, subsequent urine collected into bottle.	
Day 2	Last urine collected into bottle is starting time of Day 1	Send bottle to laboratory for UFC and creatinine

INTERPRETATION :

Normal response – Consult the reference interval provided by the laboratory. The laboratory should measure urine creatinine to assess adequacy of collection.

False positive responses – Over 24-hour urine collection. Excessive urine volume (14).

False negative responses - Inadequate 24-hour urine collection, renal impairment, cyclical Cushing's (off phase).

2.4 2-DAY LOW DOSE DEXAMETHASONE SUPPRESSION TEST (LDDST)

RATIONALE:

Patients with pseudo-Cushing's might not suppress their cortisol with 1 mg dexamethasone suppression test and require the 2 day dexamethasone regimen.

PREPARATION & PROCEDURE:

1. Exclude exogenous glucocorticoid use and medications that may induce metabolism of dexamethasone. Ensure female patients are not on oral oestrogen therapy.
2. Baseline serum cortisol and plasma ACTH to be taken prior to administration of dexamethasone.
3. Dexamethasone 0.5 mg (require total of eight tablets of 0.5 mg tablets for this test) to be given to patient (prescription of dexamethasone may be required)
4. Patient instructed to take dexamethasone 0.5 mg at ***exactly*** 6-hourly intervals.
   Option A: 9am, 3pm, 9pm, 3am (patient to set alarm clock for 3am), 9am, 3pm, 9pm, 3am, last blood test 9am (6 hrs after last dexamethasone dose)
   Option B: 8am, 2pm, 8pm, 2am (patient to set alarm clock for 2am), 8am, 2pm, 8pm, 2am, last blood test 8am (6 hrs after last dexamethasone dose)
5. Patient to present to laboratory collection centre for blood test at exactly 9am for serum cortisol for two consecutive days. Two separate request forms should be given to patient.
   1st request form "Baseline serum cortisol and plasma ACTH".
   2nd request form "2-day Dex Suppression – Day 2 serum cortisol"

Time	Procedure	Comment
Day 0 (Baseline) 8-9 am	Blood test for cortisol and ACTH (baseline) Bring completed urine collection to lab.	
Day 1: 9 am	Patient to take 0.5 mg dexamethasone	
Day 1: 3pm	Patient to take 0.5 mg dexamethasone	
Day 1: 9pm	Patient to take 0.5 mg dexamethasone	
Day 1: 3 am	Patient to take 0.5 mg dexamethasone	Patient to set alarm clock for 3am to take the Dex tablet.
Day 2: 9am	Patient to take 0.5 mg dexamethasone. Start second urine collection if requested.	
Day 2: 3pm	Patient to take 0.5 mg dexamethasone	
Day 2: 9pm	Patient to take 0.5 mg dexamethasone	

Day 2: 3 am	Patient to take 0.5 mg dexamethasone	Patient to set alarm clock for 3am to take the Dex tablet.
Day 3: 9 am (end of test)	Blood test for cortisol and ACTH (day 2)	Measure dexamethasone level if querying compliance/ absorption/ metabolism issue.

INTERPRETATION :

Normal response = Serum cortisol < 50 nmol/L

False positive responses – non-compliance, malabsorption, drugs inducing the hepatic metabolism of dexamethasone (phenytoin, carbamazepine, phenobarbital, rifampicin), drugs elevating CBG (oestrogens), chronic kidney disease.

False negative responses – nephrotic syndrome (↓CBG), renal dialysis, chronic liver disease (reduced metabolism and clearance of dexamethasone).

Sensitivity 96%, Specificity 70%.

2.5 DEXAMETHASONE-CRH TEST

RATIONALE:

Patients with pseudo-Cushing's maintain sensitivity to negative feedback with glucocorticoid and will be unable to mount a pituitary-adrenal response to CRH when pre-treated with low dose dexamethasone. Conversely, patients with Cushing's syndrome are mostly insensitive to low-dose dexamethasone suppression and will display unsuppressed cortisol levels and, in patients with a pituitary ACTH-secreting tumour, the pituitary will respond to CRH stimulation.

PREPARATION & PROCEDURE:

1. Exclude exogenous glucocorticoid use and medications that may induce metabolism of dexamethasone. Ensure female patients are not on oral oestrogen therapy.
2. Baseline serum cortisol and plasma ACTH to be taken prior to administration of dexamethasone.
3. Dexamethasone 0.5 mg (require total of eight tablets of 0.5 mg tablets for this test) to be given to patient (prescription of dexamethasone may be required)
4. Patient instructed to take dexamethasone 0.5 mg at ***exactly*** 6-hourly intervals. (1200, 1800, 2400, 0600, 1200, 1800, 2400, 0600)
5. Blood test at 0800 for cortisol and ACTH
6. Inject CRH (1 ug/kg up to 100ug) at 0800 immediately after blood test (see peripheral CRH test protocol for more details)
7. Blood test for cortisol 15 minutes after CRH

Time	Procedure	Comment
Day 1 (Baseline) 8-9 am	Blood test for cortisol and ACTH	
Day 1: 1200	Patient to take 0.5 mg dexamethasone	
Day 1: 1800	Patient to take 0.5 mg dexamethasone	
Day 1: 2400	Patient to take 0.5 mg dexamethasone	
Day 2: 0600	Patient to take 0.5 mg dexamethasone	
Day 2: 1200	Patient to take 0.5 mg dexamethasone	
Day 2: 1800	Patient to take 0.5 mg dexamethasone.	
Day 2: 2400	Patient to take 0.5 mg dexamethasone	
Day 3: 0600	Patient to take 0.5 mg dexamethasone	
Day 3: 0800	Blood test for cortisol and ACTH	Measure dexamethasone level if querying compliance/ absorption/ metabolism issue.
Day 3: 0800	Inject 1 ug/kg CRH	
Day 3: 0815	Blood test for cortisol	

INTERPRETATION :

Normal or pseudo-Cushing :
Post dexamethasone : cortisol < 38 nmol/L AND

Post dex-CRH : cortisol < 38 nmol/L

Cushing's Syndrome:
Post dexamethasone : cortisol > 38 nmol/L
Post dex-CRH : cortisol > 38 nmol/L

False positive responses – non-compliance, malabsorption, drugs inducing the hepatic metabolism of dexamethasone (phenytoin, carbamazepine, phenobarbital, rifampicin), drugs elevating CBG (oestrogens), chronic kidney disease

False negative responses – nephrotic syndrome (↓CBG), renal dialysis, chronic liver disease (reduced metabolism and clearance of dexamethasone).

Note :

- More recent studies have shown a lower specificity of the Dex-CRH test for differentiating real Cushing's from pseudo-Cushing's, (10) the above cut-off is based on Yanovski 's study. (11)

2.6 IV 4 mg DEXAMETHASONE SUPPRESSION TEST

RATIONALE:

Intravenous dexamethasone avoids potential issues of poor compliance, variable gastrointestinal absorption and metabolism of oral dexamethasone.

PREPARATION & PROCEDURE:

Patient admitted for day procedure

1. Ensure female patients are not on oestrogen therapy
2. Insertion of IV cannula
3. Samples for baseline serum cortisol to be collected at two time-points (-60mins, -5 mins) prior to dexamethasone infusion. Commence -60 mins sampling at 0830h
4. Dexamethasone infusion 1 mg/h for 4 hours (1x4 mg ampoule of dexamethasone in 500 ml Normal Saline running at 125 ml/hr) commencing at 0930h
5. Samples for serum cortisol to be collected 3, 4 and 5 hours post infusion. Ensure blood collection is taken opposite the infusion site.
6. Patient to present to the lab the following day for serum cortisol at 9:00 am and 9:30 am (unless patient remains an inpatient)

Time	Procedure	Comment
Day1	Insertion of IV cannula	
-60 minutes (Baseline, 08:30)	cortisol	
-5 minutes	cortisol	
0 minutes (09:30)	IV Dex infusion commences for 4 hours duration.	4mg dexamethasone in 500 ml Normal Saline at 125 ml/hr
+ 3 hrs	cortisol	Ensure blood collection is taken opposite the infusion site
+4 hrs	cortisol	Completion of the 4 hr Dex infusion
+5 hrs	cortisol	Patient sent home after blood test
Day 2 +23.5h (9:00)	cortisol	Patient returns for blood test
+24h (9:30)	cortisol	

INTERPRETATION :

Diagnosis of Cushing's syndrome: (2)

- Day 2 serum cortisol level (mean of +23.5h and +24h cortisol values) >130 nmol/L or >20% of baseline cortisol (Day 1 at -60 minutes)
- Sensitivity 100%, Specificity 96%
- Cushing's disease tends to partially suppress on Day 1 with rebound increase on Day 2, while ectopic ACTH patients rarely suppress during the infusion.

2.7 ACTH Dependent Cushing's Syndrome

2.7.1 HIGH DOSE DEXAMETHASONE SUPPRESSION TEST (HDDST)

RATIONALE:

Once ACTH dependent Cushing's syndrome has been established, overnight 8 mg Dexamethasone Suppression Test assists to differentiate between Cushing's Disease and Ectopic ACTH.

PREPARATION & PROCEDURE:

1. Exclude exogenous glucocorticoid use and medications that may induce metabolism of dexamethasone. Ensure female patients are not on oral oestrogen therapy.
2. Ensure patient already had baseline morning serum cortisol
3. Dexamethasone 8mg (2x 4 mg tablets) to be given to patient (prescription of dexamethasone may be required)
4. Patient instructed to take dexamethasone at 11 pm.
5. Patient to present to laboratory collection centre for blood test between 8-9am for serum cortisol and ACTH.

Time	Procedure	Comment
8 am	cortisol	Ensure patient already had baseline morning cortisol.
11pm	Take 8 mg dexamethasone tablets	Ensure strict compliance.
8-9am the next morning	cortisol	Measure dexamethasone level if querying compliance/ absorption/ metabolism

INTERPRETATION :

Decrease in serum cortisol >50% is suggestive of pituitary Cushing's disease. Most Cushing's disease patients have a cortisol < 140 nmol/L, normal subjects have undetectable cortisol post 8mg dexamethasone suppression.

HOWEVER, 10% of patients with ectopic ACTH secretion suppress with high dose dexamethasone and some patients with pituitary tumours fail to suppress. (Sensitivity 81%, Specificity 79%)

2.7.2 PERIPHERAL CRH TEST

RATIONALE:

This test may be combined with bilateral inferior petrosal sinus sampling (BIPSS) or can be performed prior to BIPSS to provide a diagnosis of Cushing's Disease along with appropriate MRI finding and suppressed high dose dexamethasone test to avoid the need for BIPSS.

PREPARATION & PROCEDURE:

1. Order CRH (100 ug)
2. Baseline weight and blood pressure
3. Patient should be supine during the test
4. Insertion of IV cannula
5. Baseline plasma ACTH and serum cortisol collection (-5 and -1 minutes)
6. CRH to be given as an IV bolus over 30-60 seconds (1 ug/kg up to a maximum of 100 μg).
7. Warned patient of potential side-effects from CRH: flushing, metallic taste in mouth, transient hypotension
8. Collect plasma ACTH and cortisol post CRH injection at +15, +30, +45, +60 and +90 minutes.

Time	Procedure	Comment
		.
-5 min	ACTH and cortisol	
-1 min	ACTH and cortisol	
0 min	IV CRH over 30-60 seconds	Warn patient of side-effects
15 min	ACTH and cortisol	
30 min	ACTH and cortisol	
45 min	ACTH and cortisol	
60 min	ACTH and cortisol	
90 min	ACTH and cortisol	

INTERPRETATION :

There is no universal agreement on blood test interval and cut off.

Pituitary Cushing's disease is more likely than ectopic ACTH production if:

- Peak ACTH increment of >50% from mean basal values (Sensitivity 86%, Specificity 90%). (8,9)
- increase in peak cortisol concentration ≥ 30% above the mean basal values (Sensitivity 61%, Specificity 70%). (8)

Note :

- CRH used in Australia is recombinant human CRH rather than Ovine CRH used in Nieman study. (3)
- Peak ACTH has higher diagnostic accuracy than cortisol.

2.7.3 INFERIOR PETROSAL SINUS SAMPLING (IPSS)

RATIONALE:

In established ACTH dependent Cushing's syndrome, IPSS assists to differentiate whether the source of excessive ACTH is central pituitary Cushing's disease or peripheral ectopic ACTH syndrome. IPSS might not be required if pituitary adenoma > 6mm AND cortisol suppressed to high dose dexamethasone AND ACTH/ cortisol rose with peripheral CRH test.

PREPARATION & PROCEDURE:

1. Book experienced interventional radiologist, discuss if patient on blood thinning agents.
2. Confirm active phase of Cushing's by late night saliva cortisol prior to the test, liaise with laboratory regarding turn-around time and alert them to the IPSS booking. Metyrapone and ketoconazole should be stopped 1 week prior to test.
3. Fast the patient from midnight for a morning procedure
4. Under radiological guidance in the Radiology Department, catheters are placed in the left and right inferior petrosal sinuses.
5. Blood is collected at -5 min and -2 min before CRH administration, from left, right petrosal catheters and peripheral vein to measure ACTH and prolactin.
6. CRH (1 ug/kg, maximum dose up to 100 ug) is given intravenously at 0 min.
7. Further samples to measure ACTH and Prolactin are collected from left and right petrosal sinus and peripheral vein at + 2, 5, 10 and 15 minutes post CRH.

Time	Procedure	Comment
- 5 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site
- 2 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site
0 min	IV CRH (1 ug/kg, up to 100 ug)	Warn patient of side-effects of flushing and hypotension
+ 2 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site
+ 5 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site
+10 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site
+15 min	ACTH and prolactin from left, right petrosal sinus and peripheral vein	1 x 4 ml EDTA tube from each site

INTERPRETATION

- A central to peripheral ACTH ratio of ≥ 2 pre CRH and / or a ratio of ≥ 3 post CRH is consistent with Cushing's disease. (5) Sensitivity and specificity 94%
- IPSS has limited utility in localization of ACTH-secreting pituitary adenomas
- Maximal IPS/ peripheral ratio is achieved at 5 minutes in 90% of Cushing's Disease, 1% achieved the maximum ratio at 15 minutes. The 2 minutes time point was found to have the best diagnostic accuracy. (5)
- If the central to peripheral ACTH ratios were elevated on at least one side, then there is no need to assess prolactin levels as petrosal sinus cannulation would have been adequate on at least one side. De Sousa et.al. found co-lateralisation of prolactin and ACTH during IPSS in Cushing's disease subjects. (12) Therefore the prolactin inferior petrosal sinus/ peripheral ratio on the ACTH non-dominant side can be low despite adequate cannulation of IPS. The prolactin IPS/P ratio should NOT be used to 1) differentiate Cushing's Disease from ectopic ACTH, 2) correct for adequacy of IPSS, 3) lateralise ACTH producing pituitary adenoma.
- If the central to peripheral ACTH ratios were not elevated on either side, the patient might have ectopic source of ACTH, or petrosal sinus was not successfully cannulated. A petrosal sinus/ peripheral prolactin ratio of ≥ 1.8 has been used to indicate adequate petrosal sinus cannulation (7)
- False results occur if patient was not in active phase of hypercortisolism at the time of testing. Late night saliva cortisol before IPSS can assist in determining whether cyclical Cushing patients are in active phase before proceeding with the test.

NOTES:

- ACTH and prolactin can be analysed using a single 4 ml EDTA tube in most centres. This vastly reduces the number of tubes and volume of blood required for IPSS and need to be discussed with the laboratory.
- Side effects of CRH includes flushing and hypotension. Rare complications during IPSS include brain stem injury, (6) deep venous thrombosis, pulmonary embolism and venous subarachnoid haemorrhage.
- Anticoagulation with heparin can reduce prothrombotic complications.

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3 Hypopituitarism

3.1 INSULIN TOLERANCE TEST

RATIONALE

To assess the integrity of the hypothalamic pituitary adrenal axis in patients with suspected secondary adrenal insufficiency

To assess the integrity of the growth hormone axis in patients with suspected growth hormone deficiency

PREPARATION AND PROCEDURE:

1. The test should not be undertaken in patients ischaemic heart disease, cerebrovascular disease, cardiac arrhythmias or epilepsy. The test should only be done with caution in an experienced unit in patients with morning cortisol < 100 nmol/L, or >70 years of age.

2. Patients should provide written informed consent prior to the procedure.

3. For HPA axis assessment, exclude exogenous glucocorticoid use. Ensure female patients are not on oral oestrogen therapy.

4. Fast (water only) and no smoking from midnight the night before the test. Omit glucocorticoids; hydrocortisone after 1600h the day before (at least 16h) and prednis(ol)one from 0800h the day before (24h).

5. Baseline weight, pulse and BP.

6. ECG.

7. Insert an 18-20g cannula with a three-way tap into an antecubital vein. Secure venous access is crucial prior to commencing the test. The cannula should both flush freely and draw easily.

8. **The dose of insulin analog should be determined by the requesting endocrinologist prior to the procedure. The table below serves as a guide only.**

Insulin doses for adults ≥18 years:

Dose Classification	Condition	Insulin dose (units/kg)
Low dose	High probability of hypopituitarism	0.1
Standard dose	BMI <30 kg/m2, non-diabetic	0.15
Insulin resistant dose	Obese (BMI >30 kg/m2) and/or metabolic syndrome with fasting glucose >5.5 mmol/L	0.2
High dose	Active acromegaly or Cushing's syndrome, type 2 diabetes	0.3

9. The insulin analog should be diluted to 10 units/mL in 0.9% saline to ensure accurate dosing.

10. Insulin analog (Novorapid, Humulin R) 100 units/ml. 0.5 mL (50 units) + 4.5 mL 0.9% saline in a 5 ml syringe. The final dose should then be drawn up in a 1 mL or 2 mL syringe according to whether the final dose is <10 units or >10 units.

12. Point of care (measured on the venous sample) and plasma glucose should be measured throughout the test, but the final determination of adequate hypoglycaemia should be made on the basis of the plasma glucose result.

13. Take baseline samples at -5 mins (Glucose, cortisol, GH) and 0 min (Glucose, cortisol, GH, ACTH), then insulin iv over 1 minute immediately following blood sampling

14. Repeat samples at 20, 30 and 40 minutes

15. If glucose has not fallen to ≤2.2 mmol/L, administer second insulin dose 50% higher than the initial dose

* Repeat insulin dosing in the event of inadequate hypoglycaemia:

If glucose remains >2.2 mmol/L and there are no hypoglycaemic symptoms at 40 min, a second dose of iv insulin should be given at 50% higher than the initial bolus.

This should represent a new time 0 minutes, with sampling at 20, 30, 40, 60, 90 and 120 minutes after the second injection.

If glucose remains >2.2 mmol/L after the second dose, a third dose of insulin double the initial dose can be considered. However, by then both patient and investigator may be more willing to abandon the procedure.

16. An oral carbohydrate solution (e.g. lemonade‡) and 50% glucose for intravenous use must be available to treat hypoglycaemia if required, **subsequent samples should still be taken after hypoglycaemia treatment**. Hydrocortisone 100 mg for intravenous use should also be available if required, however further sampling is not possible after administration. (see notes for glucose rescue)

17. A carbohydrate meal should be given at the end of the test. For patients at high risk of hypopituitarism in whom the serum glucose has been slow to recover, consider hydrocortisone 50 mg IV **at the completion of sampling**.

PROCEDURE:

Time	Procedure	Comment
-15 minutes	Glucose, cortisol, GH	
0 minutes	Glucose, ACTH, cortisol, GH, then insulin iv over 1 minute immediately following blood sampling	
20 minutes	Glucose, cortisol, GH	
30 minutes	Glucose, cortisol, GH	
40 minutes	Glucose, ACTH, cortisol, GH	If glucose has not fallen to ≤2.2 mmol/L, administer second insulin dose 50% higher than the initial dose *
60 minutes	Glucose, cortisol, GH	
90 minutes	Glucose, cortisol, GH	
120 minutes	Glucose, cortisol, GH	

INTERPRETATION:

- Adequate hypoglycaemia defined as serum glucose ≤2.2 mmol/L
- **Cortisol cut-off is the same as the cut-off specified for short synacthen test for that particular cortisol assay (refer Section 1.1 Short Synacthen Test).**
- Normal response for cortisol: peak cortisol ≥ 500 nmol/L **(or the local cortisol cut-off specified for short synacthen test)** at any time of the test
- Abnormal response for cortisol: peak cortisol < 500 nmol/L **(or the local cortisol cut-off specified for short synacthen test)** at any time of the test
- GH > 5 ug/L excludes GH deficiency, GH <3 ug/L consistent with GH deficiency. PBS criteria for treatment of adult GH deficiency in Australia is a peak of < 2.5 ug/L.

NOTES:

Glucose rescue:

Intravenous glucose should be administered in the event of severe hypoglycaemia defined as **any of the following**:

- Plasma glucose ≤1.5 mmol/L
- Altered level of consciousness
- Seizure

Initial dose recommended is 25 mL of IV 50% glucose.

If point of care glucose <3.0 mmol/L after 5 minutes, repeat IV dose (if patient is unable to ingest oral liquid) OR administer lemonade‡ 200 mL orally.

For patients with mild-moderate hypoglycaemic symptoms, rescue with an oral carbohydrate solution is unnecessary. Early rescue may blunt the stress response and result in a falsely abnormal result.

If patient has definite hypoglycaemic symptoms for >10 minutes and point of care glucose remains ≤2.2 mmol/L, administer lemonade‡ 200 mL orally.

‡ Concentration of sugars in Sprite is 10.1g/100 mL (sucrose), so approximately 5g glucose and 5g fructose per 100 ml. Concentration in Schweppes lemonade is similar at 11g/100 ml.

REFERENCES:

1. Sarlos S and Inder WJ. Selective use of the insulin tolerance test to diagnose hypopituitarism. Int Med J 2013; 43:89-93.

2. Lange M et al. An audit of the insulin-tolerance test in 255 patients with pituitary disease. Eur J Endocrinol 2002; 147:41-7.

3. Fincuane FM et al. Clinical insights into the safety and utility of the insulin tolerance test (ITT) in the assessment of the hypothalamo-pituitary-adrenal axis. Clin Endocrinol 2008; 69:603-7.

3.2 OVERNIGHT METYRAPONE TEST

RATIONALE:

- Patients with suspected secondary adrenal insufficiency due to pituitary or hypothalamic dysfunction may have a normal cortisol response to Synacthen (Tetracosactide) and require a test of the entire HPA axis. The overnight metyrapone test provides a good alternative to the ITT, particularly if there are contraindications to performing an ITT or assessment of GH status is not required.
- Metyrapone inhibits the last step (11-hydroxylation) in the synthesis of cortisol. The negative feedback inhibition of cortisol on ACTH is thereby reduced, leading to elevated ACTH and an increased 11-deoxycortisol in normal individuals.

PREPARATION & PROCEDURE:

- If patient is taking glucocorticoid replacement, the morning glucocorticoid tablets should be taken but the evening dose is **NOT TO BE TAKEN**.
- Metyrapone comes as a 250 mg capsule
- Between 11pm and midnight, patient is to have 30 mg/kg Metyrapone, rounded up to the nearest 250 mg and maximum dose 3 g.
  - e.g. an 80 kg person would take 2.5 g (10 capsules)
- Take with a glass of milk and a snack.
- Remind patient that if they forget to have the metyrapone tablets, then not to present for a blood test the following morning.
- **The patient is not to have their morning glucocorticoid tablets prior to blood test.**
- Between 0800h and 0900h, take blood sample for 11-deoxycortisol, cortisol and ACTH.
- The patient should then have their usual morning glucocorticoid medication if prescribed.

PROCEDURE:

Time	Procedure	Comment
Day 0	Withhold evening dose of glucocorticoid	
Day 0 23:00-24:00	Metyrapone 30 mg/kg (Max 3 g)	Take with a glass of milk and a snack.
Day 1	Withhold morning dose of glucocorticoid	
Day 1 08:00 – 09:00	11-deoxycortisol, cortisol and ACTH	After blood test, can take usual morning glucocorticoid medication if prescribed

INTERPRETATION:

If cortisol <200 nmol/L, metyrapone inhibition of cortisol and subsequent ACTH stimulation has been adequate (i.e., test interpretable)

11-deoxycortisol: >200 nmol/L – normal.
<200 nmol/L – secondary adrenal insufficiency

NOTES

- In a large series from Ireland[1], side effects only occurred in 7/398 patients having 576 tests. Side effects include nausea and vomiting, dizziness, nightmares.
- The risk of adrenal crisis from acute cortisol deficiency is very low, but the test should not be performed in patients with suspected primary adrenal insufficiency.
- Some centres advocate giving the patients oral hydrocortisone or cortisone acetate to take home in case of severe symptoms of acute cortisol deficiency

REFERENCES:

1. Fiad TM, Kirby JM, Cunningham SK, McKenna TJ. The overnight single-dose metyrapone test is a simple and reliable index of the hypothalamic-pituitary-adrenal axis. Clin Endocrinol 1994; 40:603-609

2. Soule S, van Zyl C, Parolis G, Attenborough S, Peter D, Kinvig S, Kinvig T, Coetzer E. The low dose ACTH stimulation test is less sensitive than the overnight metyrapone test for the diagnosis of secondary hypoadrenalism. Clin Endocrinol 2000; 53:221-227

3. English K, Inder WJ, Weedon Z, Dimeski G, Sorbello J, Russell AW, Duncan EL, Cuneo R. Prospective evaluation of a week one overnight metyrapone test with subsequent dynamic assessments of hypothalamic-pituitary-adrenal axis function after pituitary surgery. Clin Endocrinol 2017; 87:35-43.

3.3 GLUCAGON STIMULATION TEST

RATIONALE:

Glucagon is a hormone that stimulates glycogenolysis in the liver as well as ACTH and growth hormone release from the pituitary with peak GH response after 90-180 minutes. Glucagon stimulation test is recommended as the preferred alternative to ITT for diagnosis of adult GH deficiency based on its reproducibility and safety. Glucagon also stimulates adrenal production of cortisol in subjects with adequate endogenous ACTH, however this is an inconsistent stimulus and pituitary adrenal axis should be assessed using the ITT or SST instead. (1)

Glucagon may be associated with headaches or vomiting and there is a risk of late hypoglycaemia at around 3 hours. This test should not be performed in malnourished subjects.

PREPARATION:

- Test performed in the morning between 08:00 to 09:00 after fasting from midnight.
- In view of duration and potential late hypoglycaemia best performed as a Day Admission.
- Patients with established secondary adrenal insufficiency should have a single dose of hydrocortisone 20mg orally 30 minutes before commencement of the test in case of side effects post glucagon.
- Collect baseline GH, glucose.
- Give Glucagon 1 mg (1.5 mg if weight > 90 kg) by intramuscular injection.
- Take additional blood samples at 60, 90, 120, 150, 180, 210 and 240 minutes.

PROCEDURE:

Time	Procedure	Comment
Baseline	GH, glucose, IGF-1	baseline serum IGF-1 measurement must be < 12 weeks old for GH application
0 minute	IM glucagon	
60 minutes	GH, glucose	Monitor capillary glucose
90 minutes	GH, glucose	Monitor capillary glucose
120 minutes	GH, glucose	Monitor capillary glucose
150 minutes	GH, glucose	Monitor capillary glucose
180 minutes	GH, glucose	Monitor capillary glucose
210 minutes	GH, glucose	Monitor capillary glucose
240 minutes	GH, glucose	Monitor capillary glucose

In the event of symptomatic hypoglycaemia (glucose <3) following glucagon, obtain an immediate blood sample for glucose and GH followed by 25 mL of 50% IV dextrose. Treat with IV hydrocortisone if required. If point of care glucose <3.0 mmol/L after 5 minutes, repeat IV dose (if patient is unable to ingest oral liquid) OR administer lemonade 200 mL orally. All patients should have a small snack before discharge.

In the event of asymptomatic hypoglycaemia (glucose 2.5 mmol/L – 3 mmol/L), observe closely for symptoms and treat with glucose rescue and hydrocortisone as required AFTER obtaining an immediate blood sample for glucose and GH.

INTERPRETATION: (2,3,4)

Test outcomes:	Result:	Suggested comment:
Normal glucagon stimulation test	Any GH ≥ 3 ug/L.	The glucagon stimulation test was normal, the maximum stimulated growth hormone was at least 3 ug/L therefore there was no evidence of growth hormone deficiency.
Abnormal glucagon stimulation test	Maximum serum GH < 3 ug/L	The glucagon stimulation test was abnormal, the maximum stimulated growth hormone was less than 3 ug/L, consistent with growth hormone deficiency. This result fulfils the PBS biochemical requirement for growth hormone replacement in adult.

NOTE:

- The fixed dose glucagon regimen is provided above rather than the weight-based glucagon regimen (0.03 mg/kg, to a maximum of 3 mg) because the fixed dose regimen is consistent with the Endocrine Society and the AACE/ACE guidelines. (1, 5)
- The sensitivity and specificity of the glucagon stimulation test was 100% in lean subjects. (1) Recent studies suggest subjects with obesity have lower GH response and the < 3 ug/L might over diagnose GH deficiency in this group. (1, 3) The above cut-off is used to be consistent with PBS guideline. (https://www.humanservices.gov.au/organisations/health-professionals/forms/pb248)
- GH peak generally occur after120 minutes post glucagon, however some patients have been reported to peak at 60 minutes.

REFERENCES:

1. Yuen KC, Tritos NA, Samson SL, Hoffman AR, Katznelson L. American Association of Clinical Endocrinologists and American College of Endocrinology disease state clinical review: update on growth hormone stimulation testing and proposed revised cut-point for the glucagon stimulation test in the diagnosis of adult growth hormone deficiency. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2016;22(10):1235-44.

2. Gomez JM, Espadero RM, Escobar-Jimenez F, Hawkins F, Pico A, Herrera-Pombo JL, et al. Growth hormone release after glucagon as a reliable test of growth hormone assessment in adults. Clinical endocrinology. Mar 2002; 56(3):329-34.

3. Hamrahian AH et al. Revised GH and cortisol cut-points for the glucagon stimulation test in the evaluation of GH and hypothalamic-pituitary-adrenal axes in adults. Pituitary. 2016; 19:332-341.

4. Holdaway, I.M., et al., Three-year experience with access to nationally funded growth hormone (GH) replacement for GH-deficient adults. Clin Endocrinol (Oxf), 2015. 83(1): p. 85-90.

5. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1587-1609

3.4 ARGININE STIMULATION TEST

RATIONALE:

Arginine stimulates GH secretion via effects on α-receptors which influence GHRH and somatostatin secretion from the hypothalamus. In this test, arginine is infused intravenously and the response of GH is measured in peripheral blood. It is often a useful test in the neonate or young infant when GH testing is required, since it is relatively free of adverse side effects compared to other tests at this age. This test is contraindicated during acute illness, severe cardiac or renal disease, electrolyte disturbances (acidosis) or hypothyroidism.

PREPARATION:

- Test performed in the morning between 08:00 to 09:00 after fasting from midnight and continue to fast during test.
- Collect baseline GH, IGF-1 samples.
- 10% solution of L-Arginine hydrochloride in normal saline (10 g arginine per 100 ml normal saline). IV infusion over 30 minutes. Dose = 0.5 g/kg body weight (to a maximum of 30 g)
- Take additional blood samples at 30, 45, 60, 75 minutes.

PROCEDURE:

Time	Procedure	Comment
Baseline	GH, IGF-1	Monitor capillary glucose
0 minute	Commence IV Arginine infusion	Possible side effects: allergy, hypotension, flushing, nausea, headache, hypoglycemia. Monitor capillary glucose
30 minutes	GH, after cessation of infusion	Monitor capillary glucose
45 minutes	GH	Monitor capillary glucose
60 minutes	GH	Monitor capillary glucose Expected peak of GH
75 minutes	GH	Monitor capillary glucose

INTERPRETATION: (2)

Test outcomes:	Result:	Suggested comment:
Normal Arginine stimulation test	Any GH ≥ 0.4 ug/L.	The Arginine stimulation test was normal, the maximum stimulated growth hormone was at least 0.4 ug/L therefore there was no evidence of growth hormone deficiency.
Abnormal Arginine stimulation test	Maximum serum GH < 0.4 ug/L	The Arginine stimulation test was abnormal, the maximum stimulated growth hormone was less than 0.4 ug/L, consistent with growth hormone deficiency. This

		result fulfils the PBS biochemical requirement for growth hormone replacement in adult.

NOTE:

- The GH assay originally used to derive the 0.4 ug/L cut-off was established in 1998 and calibrated against the WHO 80/505 international GH standard. This assay is no longer available.
- Current commercial GH assays are calibrated against the 2nd International Standard for somatropin (a recombinant DNA-derived human GH, IS 98/574), therefore it is uncertain whether the same cut-off is applicable.

REFERENCES:

1. Cohen, P., et al., *Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop.* J Clin Endocrinol Metab, 2008. **93**(11): p. 4210-7.
2. Biller, B.M., et al., *Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency.* J Clin Endocrinol Metab, 2002. **87**(5): p. 2067-79.

3.5 GONADOTROPHIN RELEASING HORMONE STIMULATION TEST

RATIONALE:

This test assists to differentiate severe delayed puberty vs hypogonadotrophic hypogonadism. Gonadotrophin releasing hormone (GnRH, LHRH) is a hypothalamic decapeptide stimulating synthesis and rapid release of LH and FSH in the anterior pituitary. Leuprorelin (Lucrin, Abbott) is a synthetic GnRH analogue with similar action. An absent or sub-optimal response to its administration indicates hypofunction of the hypothalamus or pituitary.

PREPARATION:

- No specific preparation.
- Generally performed in the morning but may be at any time of the day.
- Leuprorelin (Lucrin, Abbott or alternative equivalent) 5,000 µg/ml is available in a multidose vial. Dose is 0.004 ml/kg given subcutaneously (e.g. 0.2 ml/50kg). Maximum dose 0.2 ml (1,000 ug).

PROCEDURE:

Time	Procedure	Comment
Baseline	LH, FSH plus E2(F)/Testosterone (M)	
0 minute	*Leuprorelin administration*	
30 minutes	LH, FSH	
60 minutes	LH, FSH	Typically peak response
120 minutes	LH, FSH	

INTERPRETATION:

Pre-pubertal peak LH response <5 mIU/L

Normal adult peak response 20-100 mIU/L

NOTES:

Patient should be observed for 5 minutes in case of allergic reaction although no such reaction has been documented.

Some historic protocols have utilised more frequent samples with no obvious benefit.

REFERENCES:

1. Kletter GB. Commentary: How Should We Diagnose and Monitor Central Precocious Puberty? Journal of Pediatric Endocrinology & Metabolism 2008; 21: 1105 - 6.

2. Ibanez L, Potau N, Zampolli M, Virdis R, Gussinye M, Carrascosa A, Saenger P & Vicens-Calvet E. Use of Leuprolide acetate response patterns in the early diagnosis of pubertal

disorders: Comparison with the gonadotropin - releasing hormone test. JCEM 1994; 78 (1): 30-5.

3. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, & Borges MF. Assessment of Basal and Gonadotropin-Releasing Hormone-Stimulated Gonadotropins by Immunochemiluminometric and Immunofluorometric Assays in Normal Children. JCEM 2007; 92(4): 1424–9.

4. Neely EK, Hintz RL, Wilson DM, Lee PA, Gaultier T, Argente J, Stene M. Normal ranges for immunochemiluminometric gonadotropin assays. J Pediatr 1995; 127: 40–6.

5. Eckert KL, Wilson DM, Bachrach LK, Anhalt H, Habiby RL, Olney RC, Hintz RL, Neely EK. A Single-sample, Subcutaneous Gonadotropin-releasing Hormone Test for Central Precocious Puberty. Pediatrics 1996; (4): 517-519.

6. Brito VN, Batista MC, Borges MF, Latronico AC, Kohek MB, Thirone AC, Jorge B, Arnhold IV, Mendonc BB. Diagnostic value of fluorometric assays in the evaluation of precocious puberty. J Clin Endocrinol Metab 1999; 84: 3539–44.

7. Street ME, Bandello MA, Terzi C, Ibanez L, Ghizzoni L, Volta C, Tripodi C, Virdis R. Leuteinizing hormone responses to leuprolide acetate discriminate between hypogonadotropic hypogonadism and constitutional delay of puberty. Fertility & Sterility, 2002; 77(3): 555-60.

4 Acromegaly

4.1 GROWTH HORMONE SUPPRESSION TEST

RATIONALE:

To diagnose acromegaly (growth hormone (GH) excess) when IGF-1 elevated or discordant with clinical presentation. Normal subjects suppress GH secretion in response to a glucose load, whereas acromegalic subjects fail to suppress GH secretion or show a paradoxical rise. Random GH has no role for diagnosis.

In post-op acromegalic patient with elevated IGF-1 or random GH ≥ 1.0 ug/L more than 12 weeks post-surgery, OGTT suppression is used to assess residual disease. This test cannot be used in patients already on somatostatin analogue. Pegvisomant interferes with many conventional GH assays, please check with laboratory beforehand.

PREPARATION:

1. The patient should fast overnight prior to the test.
2. Control hyperglycaemia prior to testing.

PROCEDURE:

1. Patient attends for blood test after 10 hr fast
2. Collect baseline glucose, GH and IGF-1
3. Give 75g oral glucose load
4. Collect Glucose and GH every 30 minutes for 2 hours

Time	Procedure/ Test	Comment
0 minute	Glucose, GH, IGF-1	
0 minute	75g oral glucose load	
30 minutes	Glucose, GH	
60 minutes	Glucose, GH	
90 minutes	Glucose, GH	
120 minutes	Glucose, GH	

INTERPRETATION:

Normal response: GH < 1.0 ug/L at any time point

A nadir GH post glucose load < 0.4 ug/L has been considered, but due to variable performance of the commercial GH assays, the 2014 Endocrine Society Clinical Practice Guideline recommended using a universal cut-off < 1.0 ug/L for acromegalic diagnosis. Nadir GH post glucose load < 0.4 ug/Lis used as the definition for post-operative remission.

NOTES:

Poorly controlled diabetes mellitus, nutritional disorders (malnutrition, malabsorption, anorexia nervosa), renal disease and liver failure are associated with acquired GH insensitivity to glucose suppression, associated with risk of false-positive OGTT results.

REFERENCES:

1. Carmichael JD, Bonert VS, Mirocha JM, Melmed S. The Utility of Oral Glucose Tolerance Testing for Diagnosis and Assessment of Treatment Outcomes in 166 Patients with Acromegaly. J Clin Endocrinol Metab 2009; 94: 523–527.

2. Katznelson L, Laws ER, Jr., Melmed S, Molitch ME, Murad MH, Utz A, Wass JA, Endocrine S. Acromegaly: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism 2014; 99:3933-3951
3. Manolopoulou J, Alami Y, Petersenn S, Schopohl J, Wu Z, Strasburger CJ, et al. Automated 22-kD growth hormone-specific assay without interference from Pegvisomant. Clin Chem. 2012;58(10):1446-56.

4.2 GROWTH HORMONE 5 POINT DAY CURVE

RATIONALE:

The 75g oral glucose tolerance test (OGTT) is commonly used to confirm the diagnosis of acromegaly after an initially elevated IGF1. However, the OGTT is not recommended in a patient with diabetes mellitus, particularly if this is poorly controlled. Growth hormone (GH) is secreted in a pulsatile fashion in normal individuals, with low-undetectable GH concentrations observed for much of the day. Day GH profiles have been used to determine a measure of mean GH across a 24h period [1,2]. The correlation between a 13 point 24 hour day curve and a 5 point GH day curve is excellent (r = 0.99) [1]. Therefore, a 5 point GH day curve is suggested as a feasible alternative to a 75g OGTT for patients with diabetes under investigation for suspected acromegaly.

PREPARATION:

The patient should fast overnight (from 10pm) prior to the test.

PROCEDURE:

1. Patient attends for blood test after 10 hr fast
2. Collect baseline GH and IGF-1 at 0800
3. Patient have breakfast and usual medication.
4. Avoid vigorous exercise or food other than the designated time points.
5. Collect sample for GH at 1000, 1200, 1400 and 1600
6. Patient have lunch after 1200 blood test

Time	Procedure/ Test	Comment
0 minute	GH, IGF-1	
1 minute	Breakfast	After baseline blood collected
120 minutes	GH	
240 minutes	GH	
241 minutes	Lunch	After 240 minutes blood collected
360 minutes	GH	
480 minutes	GH	

INTERPRETATION:

Mean GH across the 5 point profile of >1 ug/L is suggestive of active acromegaly.

REFERENCES:

1. D'Arcy R, Courtney CH, Graham U et al. Twenty-four-hour growth hormone profiling in the assessment of acromegaly. Endocrinol Diab Metab 2018; 1:e00007

2. Roelfsema F, Biermasz NR, Pereira AM et al. Optimizing blood sampling protocols in patients with acromegaly for the estimation of growth hormone secretion. J Clin Endocrinol Metab 2016; 101:2675-2682.

5 Hyperglycaemia investigation

5.1 ORAL GLUCOSE TOLERANCE TEST (OGTT)

RATIONALE:

To diagnose diabetes mellitus or gestational diabetes. Patients who have a history of bariatric surgery (including gastric band (lap band), gastric sleeve and gastric bypass) should not be booked for the OGTT. These patients are unlikely to tolerate the 300ml volume required within 5 minutes and have a high incidence of adverse events, including potentially serious hypoglycaemia. Instead these patients should have alternate means of diabetes screening.

PREPARATION:

1. The oral carbohydrate intake should be normal for three days prior to testing.

2. The patient should fast from 10 pm the day prior to the morning OGTT test (water allowed).

3. For diagnosis of gestational diabetes, the test is conducted between 26-28 weeks, but can be earlier for high risk patients.

PROCEDURE:

1. The test is performed in the morning, patient should rest during the test and may not eat or smoke, drinking water is permitted.

2. At baseline, collect blood for fasting glucose measurement. Concurrently test glucose on a glucometer if available. If results are >10.0 mmol/L, consider terminating the test after discussion with requesting doctor as per local procedure.

3. Give glucose solution 75 g orally (75 gram of anhydrous glucose or 82.5 gram glucose monohydrate), consume within 5 minutes.

4. Collect blood hourly for 2 hours. (Note that 1 hour glucose collection is unnecessary in non-pregnant subjects)

5. If the patient has any abnormal symptoms during the test (vomiting, sweating, tremors, unwell), discuss with medical personnel as per local procedure.

Time	Procedure/ Test	Comment
Baseline	Glucose (glucose testing on glucometer if available)	Consider terminating test if point of care fasting glucose >10 mmol/L as per local procedure
0 minute	75g oral glucose load within 5 minutes	
60 minutes	glucose	Not required in non-pregnant subjects
120 minutes	glucose	

INTERPRETATION:

OGTT results in the **non-pregnant patient** are interpreted as below according to WHO criteria (4):

	Plasma venous glucose (mmol/L)		
Test Outcome:	**Fasting**		**2 hrs post glucose load**
Normal glucose tolerance	≤6.0	AND	<7.8
Impaired fasting glycaemia	6.1 – 6.9	AND	< 7.8
Impaired glucose tolerance	< 7.0	AND	7.8 – 11.0
Diabetes Mellitus	≥7.0	OR	≥11.1

OGTT results in the **pregnant patient** are interpreted as below according to ADIPS criteria (1):

		Plasma venous glucose (mmol/L)			
Test Outcome:	**Fasting**		**1 hr**		**2 hrs post glucose load**
Normal	≤ 5.0	AND	< 10.0	AND	< 8.5
Gestational diabetes mellitus	5.1–6.9	OR	≥10.0	OR	8.5–11.0
Diabetes mellitus in pregnancy	≥ 7.0	OR	*	OR	≥11.1

NOTES:

*there are no established criteria for the diagnosis of diabetes based on the 1-h post-load value

The above ADIPS criteria are not used in New Zealand, gestational diabetes mellitus is diagnosed if fasting ≥ 5.0 mmol/L and 2-h glucose ≥ 9 mmol/L.

REFERENCES:

1. Nankervis A, McIntyre HD, Moses R, Ross GP, Callaway L, Porter C, Jeffries W, Boorman C, De Vries B, McElduff A for the Australasian Diabetes in Pregnancy Society. ADIPS Consensus Guidelines for the Testing and Diagnosis of Gestational Diabetes Mellitus in Australia.

2. ADPSG Consensus Panel International association of diabetes and pregnancy study group's recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33:676-682

3. HAPO Collaborative Research Group. Hyperglycemia and adverse pregnancy outcomes. New Eng J Med 2008; 358:1991-2002

4. World Health Organisation / International Diabetes Federation, Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia, 2006. (Web address: http://www.who.int/diabetes/publications/en/).

6 Hypoglycaemia investigation

6.1 MIXED MEAL TEST

RATIONALE:

To trigger a hypoglycaemic episode which can be captured with plasma glucose and insulin in patients with post prandial hypoglycaemia. This is preferred to the OGTT for capturing hypoglycaemia.

PREPARATION:

Patient to fast (may drink water) from 2400 the night before test. Avoid smoking, undue exercise and alcohol. Withhold all non-essential medications.
Patient brings the Test Meal (Similar to one which provokes hypoglycemic symptoms) or a commercial meal can be provided (see Notes below).

PROCEDURE:

1. Insert intravenous cannula - usually into cubital fossa.

2. Take baseline blood for glucose, insulin, C-peptide, proinsulin

3. Give Test Meal (provided by patient, if possible). Test meal to be similar to that which the patient reports has caused symptoms. Record test meal on work sheet.

4. Collect blood specimens for glucose, insulin, C-peptide at times 0, +30, +60, +90, +120, +180, +210, +240, +270, +300 mins. Extra specimens taken if symptoms or signs of hypoglycaemia.

5. Record any symptoms experienced by patient. If hypoglycaemic symptom occurs prior to 300 minutes, take samples for Glucose, insulin, C-peptide, proinsulin, confirm glucose < 3.0 mmol/L, then correct hypoglycaemia.

6. If Whipple's triad is demonstrated a sample should also be collected for sulphonylurea analysis and insulin antibodies. Add request for proinsulin in baseline sample and hypoglycaemic sample only

Time	Procedure/ Test	Comment
Baseline	Glucose, insulin, C-peptide	Analyse proinsulin in baseline sample and hypoglycaemic sample only
0 minute	Mixed meal	
30 minutes	Glucose, insulin, C-peptide	If hypoglycaemia occurs prior to 300 minutes, take samples for Glucose, insulin, c-peptide, proinsulin, confirm glucose < 3.0 mmol/L, then correct hypoglycaemia.
60 minutes	Glucose, insulin, C-peptide	
90 minutes	Glucose, insulin, C-peptide	
120 minutes	Glucose, insulin, C-peptide	
180 minutes	Glucose, insulin, C-peptide	
210 minutes	Glucose, insulin, C-peptide	
240 minutes	Glucose, insulin, C-peptide	
270 minutes	Glucose, insulin, C-peptide	
300 minutes	Glucose, insulin, C-peptide	

INTERPRETATION:

Inappropriate endogenous hyperinsulinaemia findings are:
Plasma glucose < 3.0 mmol/L AND both of:
Insulin ≥ 20 pmol/L (≥ 3.0 IU/L)
C-peptide ≥ 200 pmol/L (≥ 0.2 nmol/L)

Ratios of insulin and glucose should not be used.

NOTES:

A standardised 470 kcal (1966 kJ) mixed meal (71 g carbohydrate, 8.5 g fat, 20 g protein) from commercially available solid and liquid supplements was recently validated (2). For comparison, the 75-gram glucose drink in a standard oral glucose tolerance test provides 300 kcal (1255 kJ).

Sample work Sheet – MIXED MEAL TEST

NAME: UR: DOB:					DATE: LOCATION:
MINS	**ACTUAL TIME**	**MEDS. GIVEN**	**BSL**	**BLOOD TESTS**	**COMMENTS**
-5				Glucose Insulin CPep	
0		**Test meal** (provided by patient)			Test Meal Contents:
				Glucose Insulin CPep	
				Glucose Insulin CPep	
				Glucose Insulin CPep	
				Glucose Insulin CPep	
				Glucose Insulin CPep	
				Glucose Insulin CPep	
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				Glucose Insulin CPep	
				Glucose Insulin CPep	

REFERENCES:

1. Cryer PE, Axelrod L, Grossman AB et al. Evaluation and management of adult hypoglycaemic disorders: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2009; 94 (3):709-728.

2. Shankar SS, Vella A, Raymond RH et al. Foundation for the National Institutes of Health beta-Cell Project T: standardized mixed-meal tolerance and arginine stimulation tests provide reproducible and complementary measures of beta-cell function: results from the foundation for the national institutes of health biomarkers consortium investigative series. Diabetes Care. 2016;39:1602–13.
3. Berry SE, Valdes AM, Drew DA, Asnicar F, Mazidi M, Wolf J, et al. Human postprandial responses to food and potential for precision nutrition. Nature Medicine. 2020;26(6):964-73.

6.2 PROLONGED OGTT

RATIONALE:

To capture a hypoglycaemic episode in patients with post-prandial hypoglycaemia, however a mixed meal test is preferable.

PREPARATION:

1. The oral carbohydrate intake should be normal for three days prior to testing.
2. The patient should fast from 10 pm the day prior to the morning OGTT test (water allowed).

PROCEDURE:

1. The test is performed in the morning, patient should rest during the test and may not eat or smoke, drinking water is permitted.
2. At baseline, collect blood for fasting glucose measurement.
3. Give glucose solution 75 g orally.
4. Collect blood glucose, insulin and C-peptide hourly for 3 hours.
5. If the patient has any abnormal symptoms during the test (vomiting, sweating, tremors, unwell), take blood sample immediately and discuss with medical personnel as per local procedure.

Time	Procedure/ Test	Comment
Baseline	Glucose, insulin, C-peptide	
0 minute	75g oral glucose load	Take blood samples immediately if hypoglycaemic symptoms.
60 minutes	Glucose, insulin, C-peptide	
120 minutes	Glucose, insulin, C-peptide	
180 minutes	Glucose, insulin, C-peptide	

INTERPRETATION:

Inappropriate endogenous hyperinsulinaemia findings are the same for the mixed meal test and 72 hr fast:

Plasma glucose < 3.0 mmol/L AND both of:
Insulin ≥ 20 pmol/L (≥ 3.0 IU/L)
C-peptide ≥ 200 pmol/L (≥ 0.2 nmol/L)

6.3 72 HOUR FAST

RATIONALE:

To trigger a fasting hypoglycaemic episode which can be captured with plasma glucose and insulin in patients in whom samples could not be captured during spontaneous hypoglycaemia.

PREPARATION:

1. IV access inserted and patient admitted to hospital.

2. The patient is to fast (nothing to eat) and is allowed only water, black tea or black coffee for the duration of the test. Patient is to remain active during waking hours.
3. Baseline blood tests for glucose, insulin, C-peptide (add proinsulin and beta-hydroxybutyrate if hypoglycaemic episode occurs during fast)
4. Capillary blood glucose taken 3-hourly and recorded.
5. Throughout the investigation blood samples are taken **every 6 hours** for:
   - 2 ml Fluoride EDTA - glucose
   - 8 ml Serum - **ON ICE** for insulin and C-peptide.
   - If capillary glucose falls below 3.3 mmol/L, increase blood sampling frequency to hourly.

6. If there are **symptoms** of hypoglycaemia during the test:
   a. Notify the Endocrine Team
   b. Record symptoms (*eg. drowsiness, mood change, anxiety, hunger, sweating or symptoms previously experienced by patient)*
   c. Take blood samples BEFORE treating hypoglycaemia for:
      i. 2 ml Fluoride EDTA - glucose
      ii. 2x 8 ml Serum - **ON ICE** for insulin, C-peptide, and beta-hydroxybutyrate.

<u>Do not terminate the test before medical review.</u>

The medical officer may terminate the test if one of these conditions is met.

A) Symptoms and/or signs of hypoglycaemia AND plasma glucose <2.5 mmol/L measured by either plasma glucose or whole blood on blood gas analyser <u>OR</u>

B) If 72 hours have elapsed without symptoms

<u>Once decision made to terminate fast.</u>

7. Take blood samples for
   - Glucose
   - 2 x 8 ml Serum - **ON ICE** for insulin and C-peptide and beta-hydroxybutyrate, proinsulin, sulphonylurea screen and insulin antibody levels.
8. Administer 1mg glucagon IV before patient is fed and collect blood for glucose, beta-hydroxybutyrate at 10, 20, 30 minutes after glucagon.
9. Feed patient and ensure normoglycaemia

Time	Procedure/ Test	Comment
0 minute	Baseline glucose, insulin, C-peptide, (add proinsulin retrospectively only if positive 72 hr fast) Commence Fast, 4-hourly capillary blood glucose.	allowed only water, black tea or black coffee
6 hours	Glucose, insulin, C-peptide.	If capillary glucose <3.3 mmol/L, check plasma glucose If plasma glucose <3.3 mmol/L, blood sampling hourly If symptomatic AND plasma glucose < 2.5 mmol/L, terminate fast
12 hours	Glucose, insulin, C-peptide.	
18 hours	Glucose, insulin, C-peptide.	
24 hours	Glucose, insulin, C-peptide.	
30 hours	Glucose, insulin, C-peptide.	
36 hours	Glucose, insulin, C-peptide.	
42 hours	Glucose, insulin, C-peptide.	
48 hours	Glucose, insulin, C-peptide.	
54 hours	Glucose, insulin, C-peptide.	
60 hours	Glucose, insulin, C-peptide.	
66 hours	Glucose, insulin, C-peptide.	
72 hours	Glucose, insulin, C-peptide.	If asymptomatic, plasma glucose ≥ 2.5 mmol/L, terminate fast at 72 hours
Termination of fast	Glucose, insulin, C-peptide, beta-hydroxybutyrate, proinsulin, sulphonylurea screen, and insulin antibody levels. After samples taken, IV 1 mg glucagon	
10 minutes	Glucose, beta-hydroxybutyrate	
20 minutes	Glucose, beta-hydroxybutyrate	
30 minutes	Glucose, beta-hydroxybutyrate	
	Feed patient	Ensure normoglycaemia before discharge

INTERPRETATION:

Inappropriate endogenous hyperinsulinaemia (insulinoma, nesidioblastosis, post gastric bypass hypoglycaemia) findings are:

- Plasma glucose < 3.0 mmol/L AND:
- Insulin ≥ 20.8 pmol/L (≥ 3.0 IU/L)
- C-peptide ≥ 200 pmol/L
- Proinsulin ≥ 5 pmol/L
- beta-hydroxybutyrate < 2.7 mmol/L
- plasma glucose increased by 1.4 mmol/L post glucagon injection

Patterns of findings during prolonged fasting									
Symptoms and/or signs	Glucose mmol/L	Insulin mIU/L	C-peptide pmol/L	Proinsulin pmol/L	β-Hydroxy-butyrate mmol/L	Glucose increase after glucagon mmol/L	Circulating oral hypo-glycaemic	Antibodies to insulin	Diagnostic interpretation
No	< 3.0	< 3	<200	< 5	> 2.7	< 1.4	No	Neg	Normal
Yes	< 3.0	>> 3	<200	< 5	≤ 2.7	> 1.4	No	Neg (Pos)	Exogenous insulin
Yes	< 3.0	≥ 3	≥200	≥ 5	≤ 2.7	> 1.4	No	Neg	Insulinoma, NIPHS, PGBH
Yes	< 3.0	≥ 3	≥200	≥ 5	≤ 2.7	> 1.4	Yes	Neg	Oral hypo-glycaemic agent
Yes	< 3.0	>> 3	>>200	>> 5	≤ 2.7	> 1.4	No	Pos	Insulin autoimmune
Yes	< 3.0	< 3	<200	< 5	≤ 2.7	> 1.4	No	Neg	IGF
Yes	< 3.0	< 3	<200	< 5	> 2.7	< 1.4	No	Neg	Not insulin (or IGF)- mediated
Neg, negative; Pos, positive; NIPHS, noninsulinoma pancreatogenous hypoglycaemia syndrome ; PGBH, post gastric bypass hypoglycaemia.									
Table copied from Journal of Clinical Endocrinology & Metabolism, March 2009, 94(3): 709-728 – "Evaluation and Management of Adult Hypoglycemic Disorders: An Endocrine Society Clinical Practice Guideline"									

NOTES:

- Insulin, C-peptide and proinsulin clearance is reduced in renal failure, above cut-offs for these analytes might not apply.
- 75% of insulinomas are diagnosed after 24 hours fast, 90% at 48 hours.

REFERENCE:

Cryer PE, Axelrod L, Grossman AB et al. Evaluation and management of adult hypoglycaemic disorders: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2009; 94 (3):709-728.

6.4 CALCIUM STIMULATION TEST FOR INSULINOMA

RATIONALE:

Reserved for cases of suspected insulinoma that do not localize by conventional noninvasive means or if confirmation is required. The calcium stimulation test may also be useful in differentiating insulinoma from diffuse nesidioblastosis, especially if imaging is negative or inconclusive[1]. The decision to proceed with calcium stimulation testing should be made in conjunction with the operating Endocrine surgeon. The test is based on the observation that exogenous intra-arterial calcium injection stimulates the release of insulin from tumor beta cells, but not from normal beta cells[2].

PREPARATION AND PROCEDURE:

1. Discontinue calcium channel blockers (may lead to severe hypoglycaemia during test) as well as non-essential medications and those with the potential to interfere with insulin secretion by insulinoma cells (such as diazoxide), 5 half-lives prior to the procedure.
2. Overnight fast – run dextrose infusion as required to avoid hypoglycaemia.
3. Visceral arteriography into
    a. Superior mesenteric artery
    b. Proximal splenic artery
    c. Midsplenic artery
    d. Gastroduodenal artery
    e. Proper hepatic arteries
4. 10% calcium gluconate diluted to volume of 5 mL with normal saline is directly injected as a bolus into individual artery at a dose of 0.0125 mmol Ca^{2+}/kg body weight (maximum total dose for entire procedure is 11.63 mmol or 93 mg Ca^{2+}).
5. 5 mL blood samples are taken at 0, 20, 40 and 60 sec after calcium injection from the right hepatic vein for insulin levels.
6. Double check samples are labeled correctly with the time and arterial site injected with calcium.

Time	**Procedure**
Baseline	Insulin checked from right hepatic vein
0	Calcium bolus injected into selected artery, repeat procedure for each artery. 1. Superior mesenteric Proximal artery 2. Splenic artery 3. Midsplenic artery 4. Gastroduodenal artery 5. Proper hepatic artery
20 seconds	Insulin checked from right hepatic vein
40 seconds	Insulin checked from right hepatic vein
60 seconds	Insulin checked from right hepatic vein

INTERPRETATION:

A greater than 2-fold rise in right hepatic vein insulin levels from 0 at times 20, 40 and /or 60 sec is required to localize an insulin secreting tumor in that portion of the pancreas supplied by the artery studied.

Artery from which positive response achieved from calcium stimulation	**Tumour Localisation Site**
Gastroduodenal artery or superior mesenteric artery	Pancreatic head and neck region
Proximal splenic or midsplenic artery	Pancreatic body and tail region
Proper hepatic arteries	Liver metastases

If a greater than 2-fold rise in insulin levels occurs with calcium injection into more than one artery, the dominant site is used to predict tumour localization (may represent overlap in tumour arterial supply). In a large (retrospective) study[3], this correctly predicted tumour location in 84% (38 of 45 cases). False negative results occurred in 5 of 45 cases (11%) attributed to arterial anomalies/technical flaws. False positive occurred in 2 of 45 cases (4%) attributed to tumour necrosis or unexplained reasons. In a retrospective analysis of 240 patients with insulinoma at the Mayo Clinic, calcium stimulation test was performed in 25% of patients, with a sensitivity of 93% for the regionalization of functioning insulinomas[4]. Nesidioblastosis is suggested by a >2-fold rise in right hepatic vein insulin level following Ca injection in the gastroduodenal, superior mesenteric and splenic arteries but not the proper hepatic artery.

NOTES:

Procedure should be performed by experienced interventional radiologists. Hypoglycaemia can occur during the procedure.

REFERENCES:

1. Thompson SM, Vella A, Thompson GB, Rumilla KM, Service FJ, Grant CS, et al. Selective Arterial Calcium Stimulation With Hepatic Venous Sampling Differentiates Insulinoma From Nesidioblastosis. The Journal of clinical endocrinology and metabolism. 2015;100(11):4189-97.
2. Doppman JL, Chang R, Fraker DL, Norton JA, Alexander HR, Miller DL, et al. Localization of insulinomas to regions of the pancreas by intra-arterial stimulation with calcium. Annals of internal medicine. 1995;123(4):269-73.

3. Guettier JM, Kam A, Chang R, Skarulis MC, Cochran C, Alexander HR, et al. Localization of insulinomas to regions of the pancreas by intraarterial calcium stimulation: the NIH experience. The Journal of clinical endocrinology and metabolism. 2009;94(4):1074-80
4. Morera J, Guillaume A, Courtheoux P, Palazzo L, Rod A, Joubert M, et al. Preoperative localization of an insulinoma: selective arterial calcium stimulation test performance. J Endocrinol Invest. 2016;39(4):455-63.

7 Diabetes Insipidus

7.1 WATER DEPRIVATION TEST

RATIONALE:

For the diagnosis of polyuria-polydipsia syndrome.

PREPARATION:

1. Document polyuria (i.e. > 3 L/24 hours or 40-50 ml/kg/24 hours)
2. Exclude other reasons for polyuria such as hyperglycaemia and hypercalcaemia
3. Check renal function and electrolytes and pituitary function including fT4, TSH and basal cortisol
4. Check complete electrolytes and serum osmolality, copeptin (if available) and urine osmolality (i.e. 2nd urine sample after one hour) after overnight water deprivation (> 8 hours).
   - If urine osmolality is > 800 mOsm/kg, no further testing is required. In all other cases a classic water deprivation test is indicated. Note maximal urine osmolality ~1200 mOsm/kg. *** Depending on age and renal impairment, urine osmolality of > 600 mOsm/kg can be acceptable according to clinical judgment. (5)**

   Copeptin testing might be useful:
   - If copeptin levels are < *2.6 pmol/L* after overnight fluid deprivation diagnosis of central diabetes insipidus is suggested
   - If copeptin levels are ≥ *21.4 pmol/L* without prior thirsting the diagnosis of nephrogenic diabetes insipidus is likely and no further testing is required.
5. Patients should refrain from tobacco, alcohol and caffeine 24 hours prior to test.
6. Stop DDAVP 24 hours prior to test

PROCEDURE:

1. Start water deprivation at 8pm for mild polyuria (i.e. 3-5 L/24h), at 12am for moderate polyuria (i.e. 5-8 L/24h) and at 8am for severe polyuria (i.e. >8 L/24h)
2. On admission to day unit, take baseline weight, blood pressure and pulse, baseline blood test for serum osmolality, electrolytes, renal function, copeptin (if available) plus baseline urine osmolality and urine sodium
3. Repeat weight, blood pressure, pulse, urine osmolality and urine sodium hourly from 8am onwards
4. Repeat blood test every 4 hours (i.e. 8am, 12pm and 4pm) and at termination of test immediately prior to injection of DDAVP
5. Duration of water deprivation period will vary for different patients. Indication for termination of water deprivation are any of the following:
   a. - Urine osmolality plateaus (i.e. <30 mOsm/kg increase between two consecutive hourly measurements). This indicates maximal urinary concentrating ability.
   b. - Weight loss of more than 3% of baseline weight
   c. - Serum sodium levels > 150 mmol/L
   d. - Urine osmolality > 800 mOsm/kg
6. Intravenous injection of 2 µg DDAVP if termination urine osmolality is < 800 mOsm/kg
7. 1 hour after DDAVP administration: repeat blood test with serum osmolality and electrolytes plus repeat urine osmolality and urine sodium

Time	Procedure	Comment
Baseline (8am)	Weight, BP, HR serum osmolality, U+Es (± copeptin) urine osmolality, sodium	
Hourly	weight, BP, HR urine osmolality and urine sodium	
12:00	serum osmolality, U+Es (± copeptin)	In the setting of ongoing severe polyuria, more frequent checks of serum osmolality, electrolytes and renal function may be required according to the treating physician
16:00	serum osmolality, U+Es (± copeptin)	In the setting of ongoing severe polyuria, more frequent checks of serum osmolality, electrolytes and renal function may be required according to the treating physician
Termination	serum osmolality, U+Es (± copeptin)	- Urine osmolality plateaus (i.e. <30 mOsm/kg increase between two consecutive hourly measurements). This indicates maximal urinary concentrating ability - Weight loss of more than 3% of baseline weight - Serum sodium levels > 150 mmol/L - Urine osmolality > 800mOsm/kg
	IV 2 μg DDAVP	
1 hour post DDAVP	Weight, BP, HR serum osmolality, U+Es (copeptin) urine osmolality, sodium	Patient to limit fluid intake to 500-800ml for next 24 hrs if urine concentrated post DDAVP

INTERPRETATION:

	Primary polydipsia	Nephrogenic Diabetes Insipidus	Complete Central Diabetes Insipidus	Partial Central Diabetes Insipidus
Baseline copeptin (pmol/L)	≥ 5	≥ 21.4	< 2.6	< 5
Serum osmol (mOsm/kg)	< 300	> 300	> 300	> 300
Urine osmol (mOsm/kg)	> 800 (300 – 800 if chronic)	< 300	< 300	300-800
Post DDAVP urine osmol Rise (%)	< 10%	< 50%	> 50%	10-50%

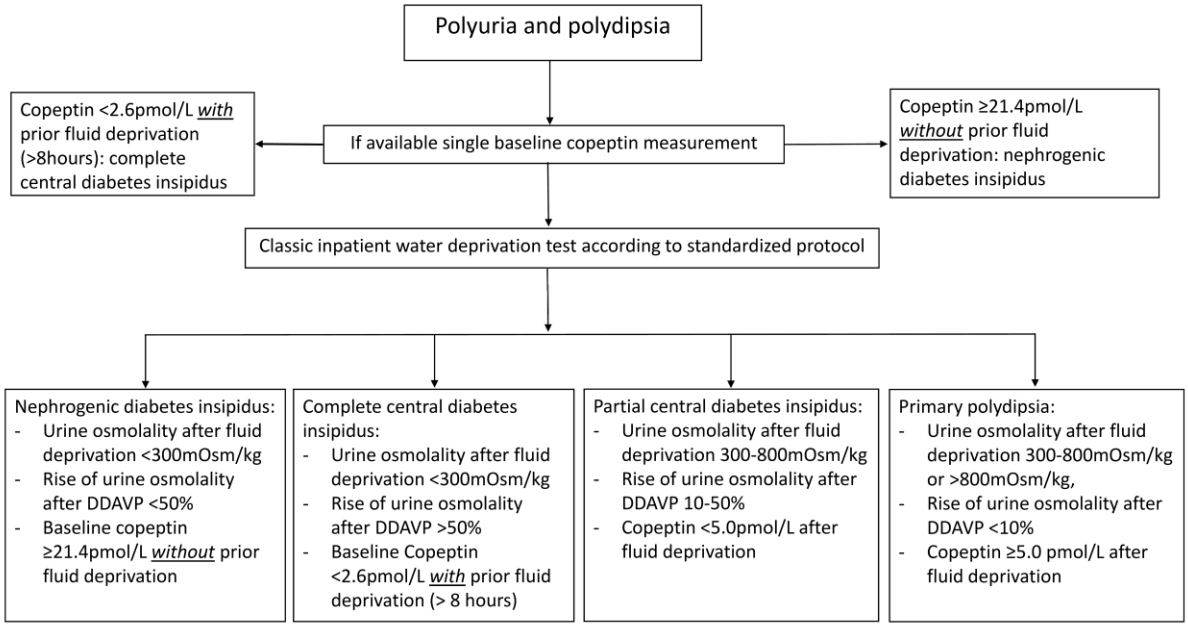

Figure: Diagnostic algorithm for the differential diagnosis of the polyuria-polydipsia syndrome (3)

NOTES:

- In the setting of severe ongoing polyuria, more frequent checks of serum osmolality, electrolytes and renal function may be required according to the treating physician.
- Hyponatraemia can occur due to excess water retention after administration of desmopressin, therefore patients should be instructed to restrict oral intake to 500-800 ml for the next 24 hours if urine is concentrated post DDAVP. (4)

- Urine concentrating ability following water deprivation declined by 20% among healthy older subjects (60-79 year vs 20 – 39 year of age). This age-related decline in urine concentrating ability is not explained by ADH response or decrease in GFR. (5)

REFERENCES:

1. Miller M, Dalakos T, Moses AM, Fellerman H, Streeten DH. Recognition of partial defects in antidiuretic hormone secretion. Annals of internal medicine. 1970;73(5):721-9.

2. Fenske W, Quinkler M, Lorenz D, Zopf K, Haagen U, Papassotiriou J, et al. Copeptin in the differential diagnosis of the polydipsia-polyuria syndrome--revisiting the direct and indirect water deprivation tests. The Journal of clinical endocrinology and metabolism. 2011;96(5):1506-15.

3. Nigro, N., M. Grossmann, C. Chiang and W. J. Inder (2018). "Polyuria-polydipsia syndrome: a diagnostic challenge." Intern Med J **48**(3): 244-253.

4. Fenske W, Refardt J, Chifu I, Schnyder I, Winzeler B, Drummond J, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. N Engl J Med. 2018;379(5):428-39.

5. Rosinger AY, Pontzer H, Raichlen DA, Wood BM, Tanner SN, Sands JM. Age-related decline in urine concentration may not be universal: Comparative study from the U.S. and two small-scale societies. American journal of physical anthropology. 2019;168(4):705-16.

WATER DEPRIVATION TEST – Worksheet

Patient Label:

Date of Test:

Patient thirsting since:

Time	BP/HR	Weight kg	Urine Vol mL/hr	Urine Na mmol/L	Serum Na mmol/L	Serum Osm mosm/Kg
08:00						
09:00						
10:00						
11:00						
12:00						
13:00						
14:00						
15:00						
16:00						
IV 2 μg DDAVP						
17:00						

7.2 ARGININE STIMULATED COPEPTIN TEST

RATIONALE:

The infusion of the amino acid arginine is a non-osmotic stimulus of the posterior pituitary in healthy adults and children. Arginine-stimulated copeptin concentrations can differentiate between patients with central diabetes insipidus and primary polydipsia with high diagnostic accuracy. (1)

PREPARATION:

- The requesting clinician must make an evaluation on the severity of the condition and likely risk to the patient of the test.
    - High risk patients: may be allowed to drink freely until 8am on the day of the test.
    - Low risk patients: must fast with no food or fluids after midnight of the night before the test. (E.g., if the test is on a Monday, patients must fast and thirst from midnight on Sunday night.)
- Patients may have their usual glucocorticoid replacement (if any) before the test.
- Patients on desmopressin should have discontinued this at least 24 hours before the test.

PROCEDURE:

1) Patient is fasted and thirsted from midnight.
2) At 8 am, obtain baseline weight, blood pressure, heart rate.
3) Take baseline blood for U+Es, plasma osmolality and copeptin
4) Set up an arginine (L-arginine-hydrochloride) infusion diluted in 500 mL of 0.9% sodium chloride solution, at a dose of 0.5 g/kg bodyweight to infuse over 30 minutes.
5) Obtain weight, blood pressure, heart rate at 30,60,90,120 minutes post arginine infusion. Call for medical review if weight loss of > 3%
6) At 60 mins after the start of infusion, take blood for U+Es, plasma osmolality and copeptin, after which the patient can eat and drink freely.

Time	Procedure	Comment
Baseline (8am)	Weight, BP, HR plasma osmolality, U+Es, copeptin	Patient is fasted and thirsted from midnight and throughout the duration of the test
0 minute	arginine infusion diluted in 500 mL of 0.9% sodium chloride solution, at a dose of 0.5 g/kg bodyweight to infuse over 30 minutes	
30 minutes	Weight, BP, HR	
60 minutes	Weight, BP, HR plasma osmolality, U+Es, copeptin	Patient can eat and drink after blood test taken
90 minutes	Weight, BP, HR	
120 minutes	Weight, BP, HR	

INTERPRETATION: (1 – 3)

Baseline copeptin level:

- >21.4 pmol/L = nephrogenic diabetes insipidus (sensitivity = 100% , specificity = 100%).
- < 2.6 pmol/L with prior fluid deprivation (> 8hrs) = complete central DI

Arginine-stimulated copeptin

- < 3.8 pmol/L without hypernatremia or nausea or vomiting = central DI, (sensitivity = 93%, specificity = 92%).
- ≥ 3.8 pmol/L to ≤ 4.9 pmol/L
    - without hypernatremia or nausea or vomiting = central DI (sensitivity = 92% , specificity = 93%).
    - with hypernatremia or nausea or vomiting, then uninterpretable, progress to hypertonic saline stimulated test
- > 4.9 pmol/L = primary polydipsia

REFERENCES:

1) Winzeler B, Cesana-Nigro N, Refardt J, Vogt DR, Imber C, Morin B, et al. Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study. Lancet (London, England). 2019;394(10198):587-95.

2) Reference Interval for fasting and water deprived adults (> 8hours) was adopted from the Mayo Clinic in house study, www. Mayoliniclabs.com.

3) Fenske W, Quinkler M, Lorenz D, Zopf K, Haagen U, Papassotiriou J, et al. Copeptin in the differential diagnosis of the polydipsia-polyuria syndrome--revisiting the direct and indirect water deprivation tests. The Journal of clinical endocrinology and metabolism. 2011;96(5):1506-15.

7.3 HYPERTONIC SALINE STIMULATED COPEPTIN TEST

RATIONALE:

For the diagnosis of polyuria-polydipsia syndrome to differentiate between primary polydipsia from central diabetes insipidus. When an individual is subjected to an osmotic stimulus through the intravenous infusion of hypertonic saline, AVP (and copeptin) secretion is stimulated in subjects with intact posterior pituitary function. Therefore, in a person presenting with polyuria-polydipsia syndrome, a low stimulated serum copeptin indicates the presence of central diabetes insipidus. Nephrogenic diabetes insipidus can often be determined by a baseline copeptin (>21.4 pmol/L) and these patients should not undergo a hypertonic saline-stimulated copeptin test. (1)

PREPARATION:

1) Exclude the following conditions which are contraindicated for this test:
   Polyuria secondary to hyperglycemia or hypercalcemia, electrolyte disorders, untreated or insufficiently replaced pituitary, adrenal or thyroid hormone deficiency, impaired renal function, nephrogenic Diabetes Insipidus, hypervolemia states (heart failure, chronic liver disease), uncontrolled hypertension, pregnancy, epilepsy, acute illness.
2) The following investigations will need to be checked a few days prior to the test to ensure the patient is suitable to be booked: paired urine and serum measured osmolality, U+E, calcium, LFTs, glucose, TFTs, 8 am cortisol
3) Withhold interfering medications as required for 24 hours prior to testing and advise patient when to re-start taking these medications: Diuretics, SGLT-2 inhibitors, Desmopressin, Carbamazepine, Non-steroidal anti-inflammatory drugs.
4) Refrain from tobacco, alcohol and caffeine 24 hours prior to the test as these substances can stimulate AVP/copeptin release.
5) Fast from midnight, water permitted until 6 am prior to procedure.
6) If a patient has adrenal insufficiency, administer their usual dose of glucocorticoid on the morning prior to the test. Stress doses of glucocorticoids may affect AVP/copeptin release. If a patient with adrenal insufficiency becomes significantly unwell during the test, the test should be terminated, and the patient administered stress dose hydrocortisone.

PROCEDURE:

1. Obtain baseline observations of: weight, blood pressure, heart rate, respiratory rate and oxygen saturation
2. Insert a cannula (20 gauge) into the antecubital vein of each arm, one for infusion and the other for blood sampling (hypertonic saline can cause thrombophlebitis and should be administered via large peripheral vein only).
3. Collect at baseline for:
   - Venous blood gas for sodium, glucose
   - plasma U+Es, albumin, osmolality
   - Urine volume and osmolality
4. Collect urine whenever urine is passed for urine volume and osmolality
5. An initial 250 mL bolus infusion of 3% saline is administered over 15 minutes (check the maximum rate as per local institution protocol), followed by continued infusion at a rate of

0.15 mL per kilogram per minute using actual body weight. Typically, allow for up to a maximum of three hours of infusion.

6. Sodium levels are to be taken every 30 minutes by venous blood gas until the target sodium of at least 150 mmol/L is achieved. Sodium levels may be monitored more frequently (i.e., 15 mins) if approaching 150 mmol/L to avoid excessive hypernatremia. Once this level is achieved, a final blood sample for sodium, serum osmolality and copeptin will be drawn and the 3% hypertonic saline infusion stopped.
7. Immediately after the hypertonic infusion being stopped, the patient is to be given water orally (30 mL per kilogram body weight) to drink as needed to thirst.
8. Within 40 minutes of the patient initially receiving the water orally, an infusion of 500 mL 5% glucose infusion over one hour will be administered.
9. The plasma sodium will be measured one hour after the commencement of the glucose infusion to ensure normalization of the sodium level (serum Na 135-145 mmol/L) prior to discharge. If the serum sodium has not normalised, allow the patient to consume any remaining oral water and repeat the plasma sodium level.

Time	**Procedure**	**Comment**
Baseline (8am)	Weight, BP, HR, oxygen saturation plasma osmolality, U+Es, albumin copeptin VBG for Na, glucose urine osmolality	Patient is fasted and thirsted from midnight and throughout the duration of the test until termination
0 minute	250 mL bolus infusion 3% hypertonic saline administered over 15 minutes (250 mL at 1000 mL/hour)	
15 minutes	Infusion 3% hypertonic saline at rate of 0.15 mL/kg/minute VBG for Na every 15 to 30 minutes Measure urine osmolality and volume whenever urine is voided.	Frequency of VBG depends on how fast and how close the previous Na approaches 150 mmol/L
Termination	plasma osmolality, U+Es, copeptin	Terminate and stop 3% saline infusion when VBG Na $\geq$ 150 mmol/L
Immediately post termination	Patient offered water orally (30 mL per kilogram body weight) to drink as needed to thirst	
40 minutes post termination	Start infusion of 500 mL 5% glucose infusion over one hour	
1 hour post dextrose infusion	Weight, BP, HR, oxygen saturation VBG for sodium	VBG Na must be normal 135-145 mmol/L prior to discharge. If Na > 145 mmol/L, continue water orally and re-test in 30 minutes

INTERPRETATION:

Copeptin:

≤ 4.9 pmol/L = central diabetes insipidus

> 4.9 pmol/L = primary polydipsia

(sensitivity of 93.2% , and specificity of 100% to discriminate between primary polydipsia and central diabetes insipidus)(1)

NOTES:

- Please ensure the rate of 3% saline is compliant with local hospital protocols.
- Adverse effects that may occur are usually mild and transient and include thirst, vertigo, headache, nausea, vomiting, malaise, shivering, and diarrhoea. If the patient is very thirsty during the test, ice-chips can be given.
- Fluid overload can occur in patients who did not have appropriate clinical and biochemical screening.
- Subjects who experience nausea and vomiting during the test have an increased copeptin response (2). This may cause a false positive elevation in copeptin in an individual with partial central diabetes insipidus because non-osmotic AVP/copeptin secretion can be preserved.
- Anti-emetic medications can affect AVP/copeptin release and should not be administered during the test.

REFERENCES:

1. Fenske W, Rerardt J, Chifu I et al. A copeptin-based approach in the diagnosis of diabetes insipidus. N Engl J Med 2018; 379:428-439

2. Brooks E, Bachmeier C, Vorster J et al. Copeptin is increased by nausea and vomiting during hypertonic saline infusion in healthy individuals. Clin Endocrinol 2021; 94:820-826

8 Thyroid

8.1 TRH TEST

RATIONALE:

The sole indication for the TRH test is the evaluation of elevated thyroid hormones (free T4 and free T3) in the setting of a normal or elevated TSH – the differential diagnosis of resistance to thyroid hormone versus a TSH secreting pituitary adenoma (TSH-oma).

The following should be considered:

1. It is essential to exclude heterophil antibody interference within the discordant thyroid function panel.
2. Once interference is excluded, and temporal pattern of discordant TFTs persists, other helpful tests include:
    a. SHBG and CTX are elevated in hyperthyroidism (TSH-oma)
    b. Alpha subunit elevated in 70% of TSH-oma (not helpful in post-menopausal women)
    c. TFT testing in first degree relatives if available (thyroid hormone resistance)
    d. **THRB gene test is available for testing of thyroid hormone resistance and is positive in 90% of RTHβ.**

PREPARATION AND PROCEDURE:

1) The patient need not be fasting but should empty their bladder immediately prior to the test.
2) TSH is collected at baseline, 20 and 60 mins after IV bolus of 200 ug of TRH over 1 minute

Time	Procedure	Comment
Baseline	TSH	
0 minute	IV bolus of 200 ug of TRH over 1 minute *	Side effects: nausea, flushing, headache, micturition urgency
20 minutes	TSH	
60 minutes	TSH	

INTERPRETATION:

In normal individuals TSH increases 4-14 fold with a mean of 8.3 fold. [1]
Autonomously secreted TSH from a TSH-oma rarely increases following TRH. [2, 3]
In the setting of thyroid hormone resistance there is a normal to exaggerated TSH response. [2]

Proposed diagnostic criteria: †
TSH-oma – < two fold elevation in serum TSH
Thyroid hormone resistance – > four fold increase in serum TSH

NOTES:

Some protocols have used 400-500 ug of TRH in adults [3]

† Absolute incremental criteria proposed include "absent response" TSH rise <2 mU/L or "reduced response" TSH rise <5 mU/L [3]. Incremental TSH response to TRH may be normal in TSH-oma patients with prior thyroid ablation [3]

8.2 T3 SUPPRESSION TEST

RATIONALE:

For investigation of suspected cases of resistance to thyroid hormone (RTH) linked to thyroid hormone receptor (THR) beta mutation. As per rationale in TRH stimulation test, analytical interference needs to be excluded first. T3 suppression test might be useful in cases where other available results are contradictory, for example a) TRH test is abnormal (TSH rise <2) and MRI pituitary is normal, b) TRH test is normal and MRI revealed pituitary adenoma (adenoma occurs in 20% of RTH), c) thyroidectomised or patients with thyroid ablation.[4] However due to cardiac side effects from T3, obtaining thyroid function tests in first degree relatives and **genetic testing for a mutation in the thyroid hormone receptor gene (identified in around 90% of RTH cases) may be considered preferential to confirm the diagnosis where possible.**

PROCEDURE:

Time	Procedure	Comment
Baseline	TSH, FT4, FT3 (Cholesterol, CK, Ferritin, SHBG)	
Day 1 -3	Patient to take Tertroxin 30ug mane, 20ug nocte (total 50 ug daily)	
Day 4	TSH, FT4, FT3 (Cholesterol, CK, Ferritin, SHBG, CTX)	If TSH suppressed, TSH-oma excluded with high certainty, no need to proceed with test
Day 4-6	Patient to take Tertroxin 30ug mane, 20ug midi, 30ug nocte (total 80 ug daily)	
Day 7	TSH, FT4, FT3 (Cholesterol, CK, Ferritin, SHBG, CTX)	If TSH suppressed, TSH-oma excluded with high certainty, no need to proceed with test
Day 7-9	Patient to take Tertroxin 40 ug mane, 20 ug midi, 40 ug nocte (total 100 ug daily)	
Day 10	TSH, FT4, FT3 (Cholesterol, CK, Ferritin, SHBG, CTX)	

INTERPRETATION:

Patient with RTH typically display partial, dose dependant suppression of baseline TSH with exogenous T3. Failure to suppress TSH to any degree is suggestive of a TSHoma. Partial, dose dependant suppression of TSH with LT3 is consistent with RTH. With short term 100 ug LT3 daily >80% reduction in TSH from baseline suggests RTH while <40% suggests TSHoma.[2]

Additional analytes may be useful to assess the peripheral tissue effects of thyroid hormones. A normal response to administration of T3 is an increase in SHBG, ferritin and CTX and a decrease in cholesterol and CK. In patients with RTH these responses are reduced, or paradoxical.

CONTRAINDICATIONS:

T3 suppression test should not be performed in patients with severe pulmonary/ cardiovascular disease/ psychiatric illness/ potential to decompensate from a short period of hyperthyroidism.

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8.3 CALCIUM STIMULATION TEST FOR MEDULLARY THYROID CANCER

RATIONALE:

The test is used in patients with mildly raised basal calcitonin levels and remaining diagnostic uncertainty to distinguish medullary thyroid carcinoma (MTC) from C-cell hyperplasia (CCH) and from (for example in MEN-1 patients) co-existing calcitonin-producing neuroendocrine tumours (NET). C-cells (like parathyroid cells) express the Ca-sensing receptor and acute increases in ionized calcium will lead to a greater increase of calcitonin from MTC vs. CCH patients (1). In patients with NET or those who have elevated calcitonin due to interfering antibodies there will be limited calcitonin increase.

PREPARATION:

1. Patient fast overnight.
2. Check electrolytes and serum calcium at baseline.
3. i.v. Cannula, 18-20g.
4. Saline flush.
5. Calcium gluconate 10% (10 - 20 ml required = 93 to 186 mg of elemental calcium).

PROCEDURE:

1. Insert cannula and flush.
2. Take baseline sample for serum calcitonin and calcium.
3. Give elemental calcium 2.3 mg/kg (0.06 mmol/kg) body weight using 10% calcium gluconate at 10 ml per minute*. (4)
4. Flush cannula.
5. Take samples at 1, 2, 3, 5 and 10 and 15 minutes for calcitonin and calcium.
6. Send immediately on ice to the lab for centrifugation and freezing.

*70 kg patient should be infused with 161 mg or 4.2 mmol of elemental calcium. This amount corresponds to 18.4 ml of the 10% Ca gluconate solution

INTERPRETATION:

- In a study of healthy volunteers (95th percentile of basal calcitonin values 5.0 pg/ml in males and 5.7 pg/ml in females) 95th percentile maximally stimulated calcitonin values were 131 pg/ml in men and 90 pg/ml in women (2).
- In a study of >100 patients with MTC (n=42), RET gene mutation carriers (n=14), multinodular goitre (n=69) and healthy volunteers (n=16), basal calcitonin values >68 pg/ml in males and >18.7 pg/ml in females and stimulated calcitonin values >1,620 pg/ml in males and >184 pg/ml in females had the highest accuracy to distinguish MTC cases from CCH and normal (4).

NOTES:

Rapid calcium infusion can lead to vasodilatation and arrhythmias - cardiac arrest after iv calcium stimulation in a healthy young man without known cardiac disease has been reported (3). While larger case series have not reported serious adverse effects, use with caution and consider cardiac monitoring, and avoid in patients with significant cardiac disease.

More transient side effects lasting up to 15 minutes include flushing sensation/ feeling of warmth (98%), facial/ extremity paraesthesia, altered gustatory sensation (20%).

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9 Phaeochromocytoma

9.1 CLONIDINE SUPPRESSION TEST

RATIONALE:

To exclude the diagnosis of phaeochromocytoma / paraganglioma (PPGL) in patients with hypertension and borderline raised catecholamines or catecholamine metabolites. The test intends to discriminate patients with mildly elevated test results for plasma normetanephrine due to increased sympathetic activity from patients with elevated test results due to a PPGL. Alternate causes for elevated plasma metanephrines (e.g. medications, collection procedure) must be excluded prior to clonidine suppression test. The clonidine suppression test is only recommended in patients with equivocal biochemistry on initial testing and in situations where the diagnosis remains uncertain. Clonidine can cause hypotension and is contraindicated in frail patients with a history of hypotensive episodes/ severe coronary/ carotid disease.

PREPARATION:

1. Obtain the clonidine from pharmacy:
2. Clonidine hydrochloride 100 ug or 150 ug tablets for oral administration
3. Test Dose = 300 ug orally (4.3 ug / kg) (1, 2)
4. Stop sympatholytic medications (e.g. beta blockers) for at least 48 hours before the test.
5. No paracetamol, diuretics or tricyclics anti-depressants for 5 days. Medications including diuretics, tricyclic antidepressants and β-blockers are known to interfere with noradrenaline responses to the clonidine suppression test
6. No smoking or caffeine for 24h
7. Fast overnight.
8. Quiet environment.
9. Cancel test if baseline blood pressure is <110/60 mmHg or in volume-depleted patients. Profound hypotensive responses to clonidine can also occur in patients taking other antihypertensive medications.

PROCEDURE:

Time	Procedure	Comment
-30 minutes	Insert cannula	Patient to lie supine during the test where possible and must rest in the supine position rest for 30 minutes prior to baseline measures.
Baseline	Collect 2 samples for plasma metanephrines at 5 minutes apart. Record blood pressure and pulse.	Two baseline blood samples taken on ice.
0 minute	Give 300ug clonidine orally.	
+60 minutes	Record blood pressure and pulse.	
+120 minutes	Record blood pressure and pulse.	
+180 minutes	Collect blood for plasma metanephrines. Record blood pressure and pulse.	Blood needs to be taken ice.

INTERPRETATION:

Normally, by activating α2-adrenoceptors in the brain and on sympathetic nerve endings, clonidine (an α2-adrenoceptors agonist) suppresses catecholamine release by sympathetic nerves in patients without PPGL. However, due to autonomous tumoral secretion of catecholamines, PPGL are not influenced by clonidine-induced suppression of the sympathetic nervous system (1).
An abnormal test result indicating a PPGL includes an elevation of plasma normetanephrine at 3 h after clonidine administration and a less than 40% decrease in levels compared with baseline (2). In this retrospective analysis (2) of 48 patients with and 49 patients without PPGL who underwent clonidine suppression testing, a positive result with normetanephrine (both elevated plasma concentration after clonidine and lack of suppression) had a sensitivity of 96% and a specificity of 100%. If there was either only an elevated plasma concentration after clonidine or a lack of suppression of normetanephrine, sensitivity remained 96% but specificity dropped to 67% to 96%. Noradrenaline response to clonidine suppression was less sensitive and specific (2).

NOTES:

Hypotension and sedation can occur with clonidine.

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10 Appendix

10.1 URINE 5 HIAA PATIENT INSTRUCTIONS

5-hydroxyindoleacetic acid (5-HIAA) is the primary breakdown product of serotonin, a chemical substance that transmits messages between nerve cells. Serotonin helps to constrict blood vessels, participates in the wake-sleep cycle, and affects mood. Your doctor may requests urine 5-HIAA and/or serotonin when you have symptoms such as flushing of the face, diarrhoea, and/or wheezing.

Before the test

Discuss with your doctor

- Some medications interfere with the measurement of 5-HIAA. If you are taking any medications, including non-prescription over-the-counter medications, cough syrups, nutritional supplements and complimentary medicines, you should check with your doctor to see if any need to be temporarily withheld or changed to alternatives prior to the test. In particular, drugs used for treatment of depression, psychosis, anxiety, nausea, and migraines, might affect your test result. DO NOT STOP ANY MEDICATIONS UNLESS INSTRUCTED TO DO SO BY A MEDICAL PROFESSIONAL.

What food should I avoid?

- It is important that you AVOID certain foods or drinks that will interfere with the testing process.

- For 48-HOURS (2 days) BEFORE AND DURING the urine collection of a 24-hour urine specimen, DO NOT eat the following foods or any food or drinks containing:

Fruit	**Vegetables**	**Nuts**	**Supplements**
– Bananas – Kiwifruit – Pineapple – Plums	– Avocado – Tomato (including sauces and soups)	– All nuts – Nut containing products such as peanut butter and biscuits or snacks containing nuts	– Supplements containing serotonin

- For 24-HOURS (1 day) BEFORE AND DURING the urine collection of a 24-hour urine specimen, DO NOT drink beverages or eat foods containing **alcohol**.

- You will be given one sterile specimen collection container by your doctor or pathology collection centre.
- Write your full name and date of birth on the container.
- The bottle contains a preservative solution which ensures accurate test results.
- *DO NOT discard the solution in the specimen collection container, tip the bottle or allow the solution to come into contact with your clothing, skin or eyes and during the test.*
- *DO NOT urinate directly into the container as the preservatives used may cause injury.*
- *If you come into contact with the solution, rinse well with cool water.*

During the test

<u>Day 1</u>

- Empty your bladder first thing when get up in the morning but DO NOT collect this first urine. The first urine should be passed into the toilet and flushed as usual.
- Write the time of your first urination on the specimen collection container as **Date and Time Commenced**.
- All urine passed in the next 24 hours needs to be collected in the specimen collection container.

- To make collection easier, please use a clean disposable plastic container and once collected, pour the urine into the specimen collection container. Rinse the disposable plastic container in plain water after use.
- Swirl the container to mix the urine with preservative after each collection.
- Keep the specimen collection container in the refrigerator or a cool, dark place during collection.

Day 2

- Collect all urine up until 24-hours after the collection began. For example, if you commenced at 8:00 am Monday, the collection should continue to 8:00 am Tuesday.
- It is important to completely empty your bladder exactly 24 hours after the collection began and add this last urine to the collection bottle, even if you do not need to pass urine at this time.
- Write the time of your final urination on the specimen collection container as **Date and Time Completed**.
- Screw on the lid tightly and make sure that the container does not leak.
- Return the test request and specimen collection container as soon as possible after the last collection to your pathology collection centre. Collection centre locations are on the back of your request forms.

IF ANY URINE IS LOST OR CONTAMINATED WITH FAECES DURING THE TIMED COLLECTION, STOP THE COLLECTION. A NEW SPECIMEN COLLECTION CONTAINER WILL BE REQUIRED AS IT IS IMPORTANT THAT ALL URINE IS COLLECTED FOR EXACTLY 24 HOURS.

10.2 URINE 5 HIAA DOCTOR INSTRUCTIONS

Patients should have their diet and medication review by the test requester before undergoing 5-HIAA urine collection.

Diet

The following foods contain quantities of serotonin which may significantly elevate the results of 24 hour urine collections for 5-HIAA.
Patients should **avoid alcohol for 1 day before and 24 hours during** the collection. Patients should avoid eating the foods below **2 days before and 24 hours during** the collection:

Plantain, Banana, Walnuts, Hickory Nuts, Pineapple, Plums, Tomatoes, Kiwifruits, Avocadoes.

Patients should be advised **not** to restrict their salt intake. A low salt diet (1.2 g/day) raises 5-HIAA levels ~ 50% above the levels seen in a high salt (14 g/day) diet. Australian adults typically eat ~ 5.5 g of salt per day.

Medications

Encourage patients to disclose all medications as well as supplements, syrups, ointments and other substances which they might not normally consider medications.

5-HTP (5-Hydoxytryptophan) supplements are widely available over the counter as a pre-cursor to serotonin commonly advertised to improve mood, sleep and brain function. The recommended dose varies widely, as does the effect on 5-HIAA levels in individual patients. Patients taking 5-HTP 100mg nocte had an up to 22-fold increase in 5-HIAA levels. We recommend discontinuing 5-HTP supplements **for at least 14 days prior to testing.**

Consult testing laboratory for list of interfering medications. **Paracetamol** can interfere with some assay.
Many drugs including antidepressants, antipsychotics, anxiolytics, antiemetics, antimigraine drugs are known to affect the serotonin system. In particular, Monoamine oxidase inhibitors and serotonin reuptake inhibitors may theoretically alter 5-HIAA secretion. However, in practice there is little available data to show false elevations caused by these agents. As these agents are often necessary treatments and may be difficult to rapidly and/or easily stop, we recommend initial testing be performed on the patient's usual psychiatric medications. If 5-HIAA results while on therapy are above the reference interval, consideration for weaning the agents and recollection can be considered

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PathWest

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St Vincent's Hospital (Melbourne)

Westmead Hospital

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Version	Date	Author/s	Comments
1.0	2017	HEDT Working group	
1.5	9/2018	HEDT Working group	Open for comments on ESA and AACB website
1.6	3/2020	HEDT Working group	Modified after feedback from members
1.8	8/2021	HEDT Working group	Inclusion of borderline zone for short synacthen test, hypertonic saline stimulated copeptin test, arginine stimulated copeptin test. GH 5 point curve. 5 HIAA collection instruction.
1.9	12/2021	HEDT Working group	Modified after ESA feedback.