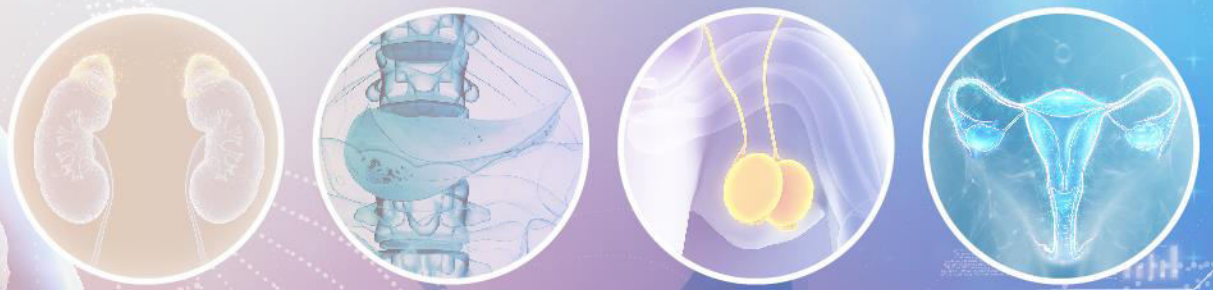
First Edition 2024

PRACTICAL GUIDE TO

ENDOCRINE DYNAMIC TESTS

KKM Endocrine Subspecialty Service



5th December 2024, Thursday

8:30am - 4:30pm

Auditorium, Academy of Medicine, Putrajaya

MEMS
Established 1981

Malaysia Endocrine & Metabolic Society

Ministry of Health Malaysia

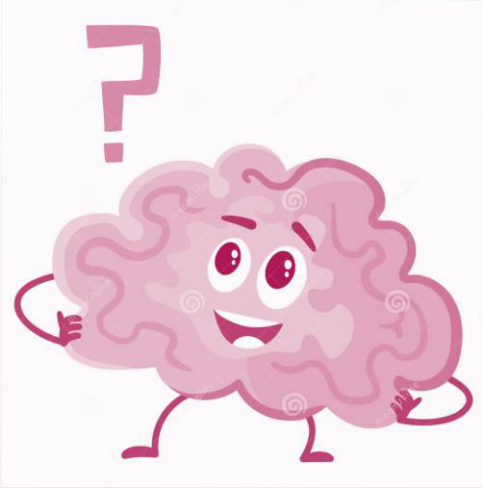
Endocrine Dynamic Tests

Evidence based

Definition of dynamic tests

Best practice

References



Interpretation

Information

Assays and units

Resources

Old vs New

Methods

First Edition 2024

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Malaysia Endocrine & Metabolic Society

Ministry of Health Malaysia

The Highlights

Practical Guide to Endocrine Dynamic Tests

5th December 2024, Thursday

8:30am - 4:30pm

Auditorium, Academy of Medicine, Putrajaya

Contents

- 12 CHAPTERS
  - Indication/contraindication
  - Specimen bottles
  - Preparation
  - Methods
  - Precaution
  - Interpretation
- Appendices
  - Results form
  - List of hospitals for test referrals
- References

1. Growth Hormone Suppression Test for Acromegaly

- 14th Acromegaly Consensus
- diagnosis of acromegaly can be confirmed in a patient with typical clinical signs and symptoms having an insulin-like growth factor-1 (IGF-1) >1.3-times the upper limit of normal (ULN) for their age

INDICATIONS

- Confirmatory test for diagnosing acromegaly when the IGF-1 level is discordant with the clinical presentation.
- OGTT might be helpful in evaluating post-surgery patients with borderline IGF-1 levels and clinical signs of disease activity.

Giustina A, Biermasz N, Casanueva FF, et al. Consensus on criteria for acromegaly diagnosis and remission. Pituitary. 2024;27(1):7-22.

2. Short Synacthen® Test (SST)

- Standard dose (250 µg) SST
- Low dose (1 µg) SST
- SST for congenital adrenal hyperplasia in adults

Assay	Serum cortisol level 30-minutes cut-off to indicate AI (nmol/L)
Advia Centaur (Siemens)	446
Architect (Abbot)	430
Modular Analytics E170 (Roche)	Females: 524 Males: 574
Access (Beckman)	459
Immulite 2000 (Siemens)	474

3. Cortisol Day Curve on Hydrocortisone

INDICATION

- To determine the concentration of cortisol throughout the day in patients on hydrocortisone replacement therapy who are suspected of having inadequate glucocorticoid replacement.

- Cortisol day curve on cortisol lowering drug – not practiced anymore
- 2 methods of performing the tests
- validity of this method for assessing the quality or sufficiency of glucocorticoid replacement remains controversial.
- may offer useful information for patients suspected of having inadequate glucocorticoid replacement

4. Anterior Pituitary Stimulation Test

- Insulin tolerance test (ITT)
- Glucagon stimulation test (GST)
- Gonadotrophin-releasing hormone (GnRH)/Luteinising hormone releasing hormone (LHRH) test
- Thyrotrophin releasing hormone (TRH) stimulation test

ITT

Interpretation

- The test cannot be interpreted unless hypoglycaemia (< 2.2 mmol/L) is achieved.
- Adequate cortisol response is defined as a rise to >500 nmol/L or depending on the assay's specific cutoff points (Refer Chapter 2).
  - Patients with impaired cortisol response between 400-500 nmol/L, will only need steroid cover for major illnesses or stress.
  - All other patients with subnormal responses require hydrocortisone replacement.
- Adequate GH response is a rise of >5 µg/L (>15 mU/L).
  - Peak serum GH levels ≤5 µg/L at any time point during the hypoglycaemic phase of the test is diagnostic of adult GH deficiency.
  - In children, a rise of >10 µg/L (>30 mU/L) is considered normal.

Sensitivity and specificity

- 5-15% of normal subjects will show a suboptimal cortisol response (cortisol level <500 nmol/L).
- In 20% of normal children, GH responses are reduced.

GST

Interpretation

- Plasma glucose usually rises and peaks at around 90 minutes and gradually declines. This is not used to interpret the test.
- Peak GH levels occur between 120-180 minutes of the IM glucagon, peaking to >3 ng/ml (or 3 µg/L).
- An adequate GH response is a rise of GH to >3 ng/ml (or 3 µg/L).
- The test is 97% sensitive and 88% specific.
- Studies suggest that subjects with overweight/obesity have a lower GH response, and the 3 ng/ml cut-off might over diagnose GH deficiency in this group.
  - In patients with overweight/obesity, a cut-off of 1 ng/ml gives the best sensitivity and specificity, and may reduce the overdiagnosis of GH deficiency.

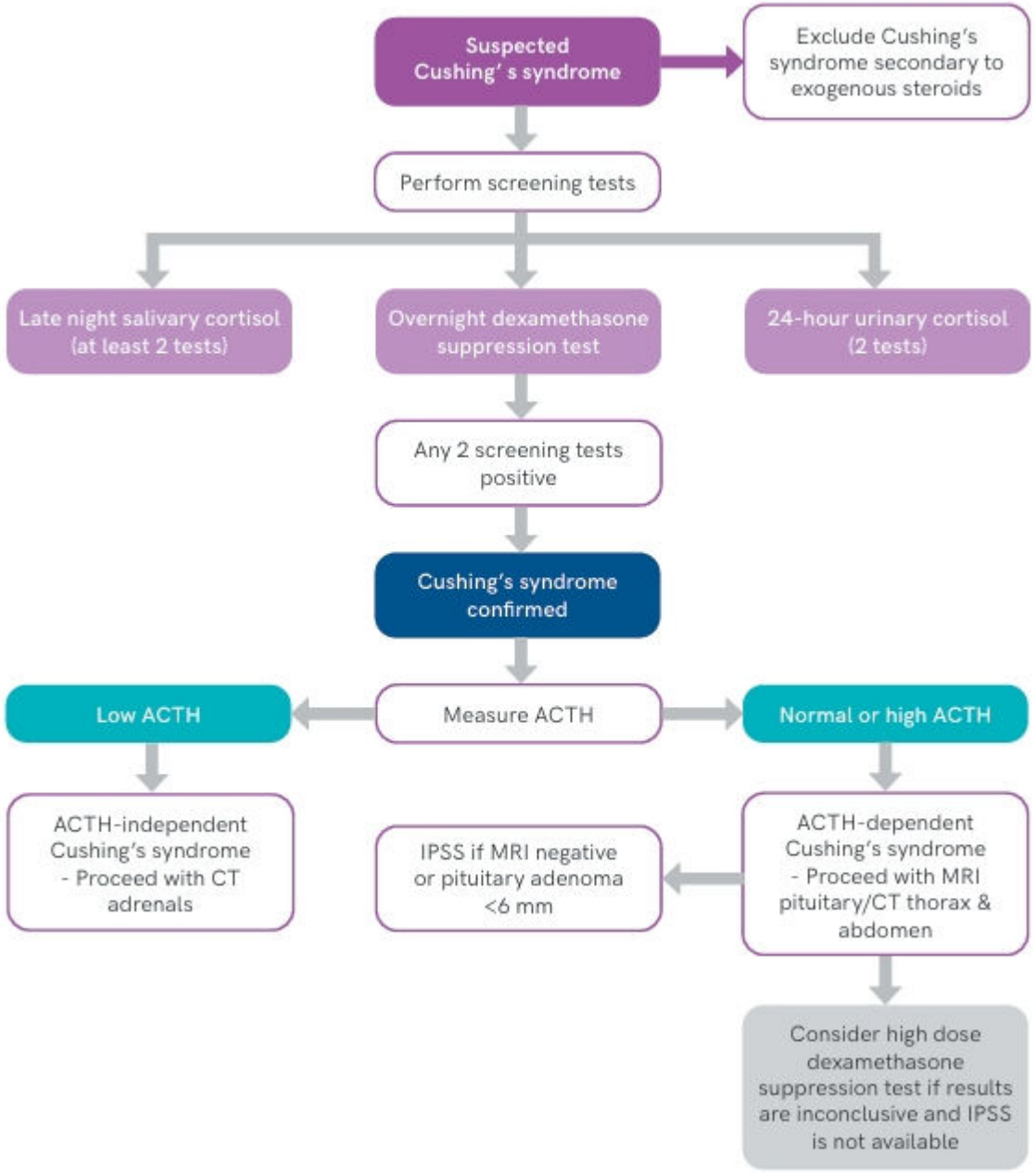
Note: The reliability of the GST for diagnosing GH deficiency in patients with glucose intolerance and frank diabetes remains unclear

5. Tests for Cushing's Syndrome

- Approach to suspected endogenous Cushing's syndrome
  - 3 screening tests

Condition	Preferred test
Pregnancy or on oral contraceptive pills (OCP)	24-hour urine free cortisol measurement
On anti-epileptic medications and rifampicin	
Cyclical Cushing's syndrome	Late night salivary cortisol
Adrenal incidentaloma	ODST
Renal impairment	
Non-neoplastic hypercortisolism/pseudo-Cushing's syndrome in: • **Patients with obesity** • **Patients who are alcoholics** • **Patients with depression & other psychiatric conditions** • **Patients with poorly controlled diabetes**	Either: LDDST + corticotropin releasing hormone (CRH) [Dex-CRH] • CRH is injected within 2 hours of the last LDDST dose. • The cortisol level is checked after 15 minutes. • A cortisol response of >38 nmol/L is suggestive of Cushing's syndrome.
	Desmopressin test • Intravenous (IV) Desmopressin 10 µg is injected. • Adrenocorticotropic hormone (ACTH) response within 30 minutes is recorded. • Pituitary Cushing's syndrome is diagnosed based on baseline serum cortisol >331 nmol/L and an absolute ACTH increase of >4 pmol/L (sensitivity 90.3% and specificity 91.5%).
	Note: The interpretation of both tests varies considerably and CRH is not available worldwide.

Figure 5-1 Algorithm for diagnosis of Cushing's syndrome



Time/Day	Day 1	Day 2	Day 3
	Oral dexamethasone 0.5 mg	Oral dexamethasone 0.5 mg	Serum cortisol
8-9 a.m.	-	-	✔
12 p.m.	✔	✔	-
6 p.m.	✔	✔	-
12 a.m.	✔	✔	-
6 a.m.	✔	✔	-

Dosing could also start from 6 a.m. or 9 a.m., 6 hourly for 48 hours. Cortisol should be taken within 2-6 hours after the last dose of dexamethasone.

Time/Day	Day 1		Day 2	Day 3
	Serum cortisol	Oral dexamethasone 2 mg	Oral dexamethasone 2 mg	Serum cortisol
8-9 a.m.	✔	-	-	✔
12 p.m.	-	✔	✔	-
6 p.m.	-	✔	✔	-
12 a.m.	-	✔	✔	
6 a.m.	-	✔	✔	-

ACTH measurement

Interpretation

ACTH results	Interpretation	Proceed with
<1.1 pmol/L (5 ng/L)	ACTH-independent Cushing's syndrome	CT adrenals
>4.4 pmol/L (20 ng/L)	ACTH-dependent Cushing's syndrome	MRI pituitary/chest x-ray/ CT thorax-abdomen
ACTH 1.1-4.4 pmol/L (5-20 ng/L)	Less discriminatory	Other tests like the HDDST may be helpful

pg/ml = ng/L. ACTH levels and normal ranges should be determined at individual centres as it depends on the sensitivity and specificity of the assays used.

Inferior petrosal sinus sampling (IPSS)

Time (minutes)	Site	ACTH	IPS/P ACTH	Prolactin	IPS/P Prolactin
0	R				
	L				
	P				
2	R				
	L				
	P				
5	R				
	L				
	P				
10	R				
	L				
	P				

R, Right IPS catheter; L, Left IPS catheter, P, peripheral line; IPS/P, ratio of R+L to P.

Table for IPSS

6. Tests for Female Infertility

- Screening for ovulation
- Progesterone challenge test

7. Tests for Male Hypogonadism/Infertility

- Human chorionic gonadotropin (hCG) stimulation test
- Tests for monitoring men undergoing spermatogenesis induction

8. Tests for Hypoglycaemia Disorders

- 72-hour fast/prolonged supervised fast
  - determine the cause of suspected spontaneous hypoglycaemia.
  - CBG is ≤2.5 mmol/L or patient is symptomatic at ≤3.0 mmol/L
- Mixed-meal test
  - suspected post-prandial hypoglycaemia
  - CBG is <3.3 mmol/L, obtain venous blood samples for plasma glucose, serum insulin mand C-peptide (sensitive cut-off value for meal-induced hypoglycaemia during OGTT or a mixed-meal test)

Interpretation

Symptoms, signs or both	Glucose (mmol/L)	Insulin (μU/mL)	C-peptide (nmol/L)	B-hydroxybutyrate (mmol/L)	Glucose increase after glucagon (mmol/L)	Diagnostic interpretation
No	<3	<3	<0.2	>2.7	<1.4	Normal
Yes		>>3	<0.2	≤2.7	>1.4	Exogenous insulin
		≥3	≥0.2	≤2.7	>1.4	Insulinoma, NIPHS, PGBH
		≥3	≥0.2	≤2.7	>1.4	OGLD*
		>>3	>>0.2	≤2.7	>1.4	Insulin autoimmunity**
		<3	<0.2	≤2.7	>1.4	IGF
		<3	<0.2	>2.7	<1.4	Not insulin (or IGF)-mediated

9. Tests for Primary Aldosteronism

- Screening test: Aldosterone renin ratio (ARR)
- Plasma aldosterone concentration (PAC) (pmol/L)

Plasma renin activity (PRA) (ng/ml/hour) or Direct renin concentration (DRC) (mU/L)

- Interpretation*
- ARR = PAC (pmol/L) / DRC (mU/L)
  - < 25 – negative (PA is highly unlikely)
  - 25 - 35 - indeterminate
  - > 35 - positive (PA highly likely)

** According to IDS - iSYS® automated assay system*

- Primary aldosteronism can be excluded if PAC is < 170 pmol/L (6 ng/dl) regardless of renin value

- Confirmatory tests
  - Saline suppression test (SST)

Interpretation

Aldosterone levels post-SST	Interpretation
< 5 ng/dl (140 pmol/L)	Normal
5-10 ng/dl (140-280 pmol/L)	Indeterminate
>10 ng/dl (280 pmol/L)	Confirmed primary aldosteronism*

**Aldosterone > 170 pmol/L (6ng/dL) if patient in seated position.*
All cut off values may differ according to the assays used. Please refer to the local laboratory reports.

- Fludrocortisone suppression test (FST)

Interpretation

Only if the serum potassium is normal, plasma renin is suppressed, and plasma cortisol is not higher at 10 a.m. than at 7 a.m.

- Normal response: Serum aldosterone is suppressed - <170 pmol/L (<6 ng/dl).
- Primary aldosteronism: Serum aldosterone is not suppressed - >170 pmol/L (>6 ng/dl).

All cut off values may differ according to the assays used. Please refer to the local laboratory reports.

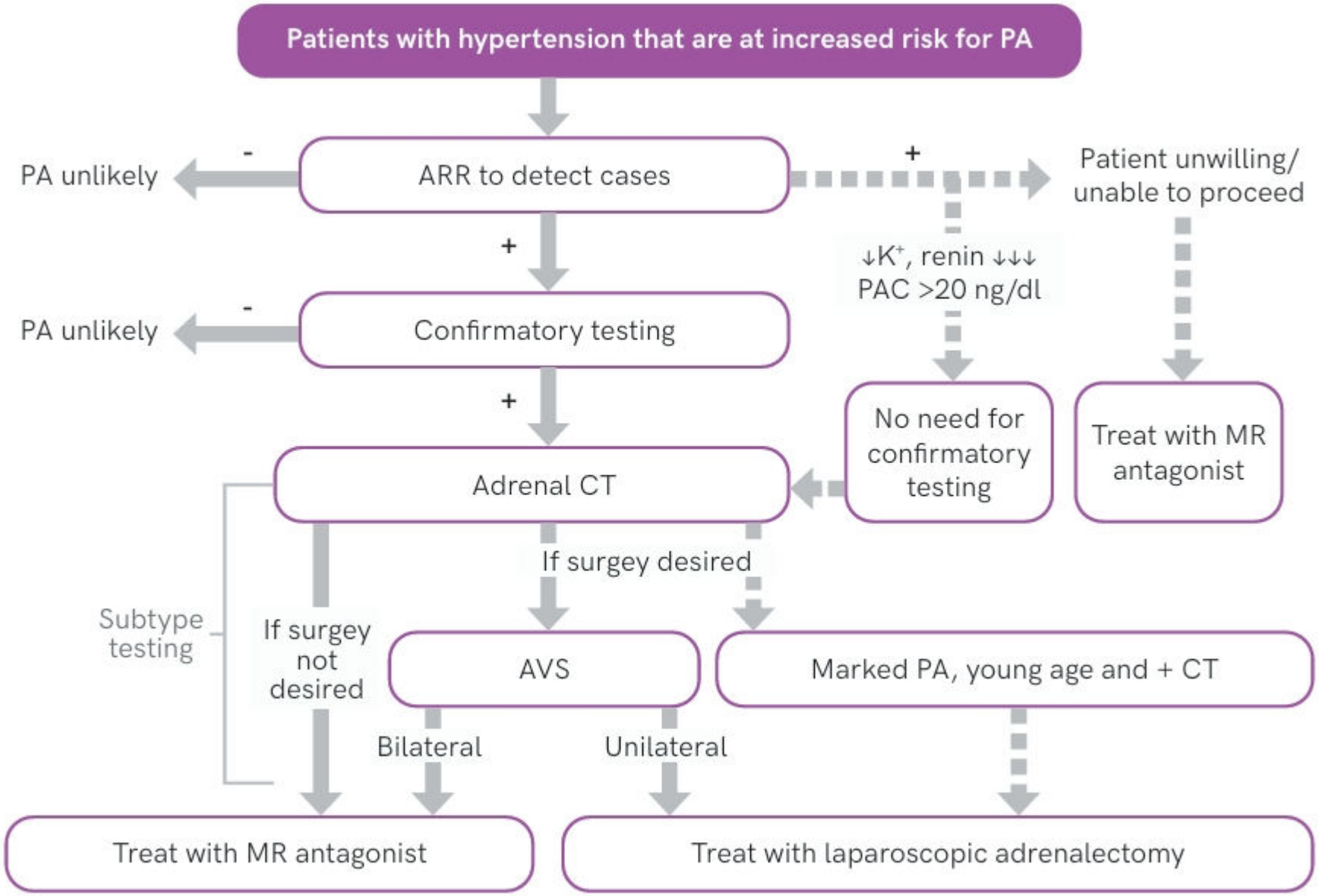
- Subtype testing
  - AVS

Lateralisation and selectivity index in adrenal venous sampling

	Lateralisation Index	Selectivity Index
ACTH Stimulation	≥4.0	≥5.0
Non-ACTH Stimulation	≥2.0	≥2.0

- Contralateral suppression (CS) ratio

Figure 9-1. An algorithm for the detection, confirmation and subtype testing of primary aldosteronism



PA, primary aldosteronism; CT, computed tomography; MR, mineralocorticoid antagonist; AVS, adrenal venous sampling.
Adapted from Funder JW, et al. J Clin Endocrinol Metab, 2008. Creative Commons CC-BY-NC-ND license.

10. Levothyroxine Absorption Test

INDICATION

- To investigate the cause of persistently elevated thyroid-stimulating hormone (TSH) in primary hypothyroid patients despite being on an adequate dose of levothyroxine (LT4) therapy. This test may help the clinician differentiate between non-compliance with LT4 and issues with LT4 absorption (true malabsorption).

Interpretation 1

The % of LT4 absorption is calculated by using the formula =
[(peak Δ total T4 or FT4 × volume of distribution [Vd] (dl) ÷ administered dose of LT4 (µg)] x 100, where Vd is 4.42 x BMI (kg/m^2).

- >60-80% absorption is considered normal and rules out LT4 malabsorption.

Interpretation 2

Adequate absorption of LT4 shows incremental increase in FT4 at:

- 120 minutes of >54% (+3%).
- 240 minutes of >60% from baseline.

Caveats

- Before proceeding with this test, the biological causes of LT4 malabsorption should be evaluated as listed below.

Causes of LT4 malabsorption or change in metabolism	**Gastrointestinal disease**	• Celiac disease • Lactose intolerance • Vitamin B12 deficiency • Intestinal infections (Giardia lamblia)	• Liver diseases • Pancreatic diseases • Previous gastrointestinal surgery/jejunoileal bypass • Short bowel syndrome
	Medication interference	• Cholestyramine • Colestipol • Aluminum hydroxide-containing antacids • Ferrous sulphate • Sucralphate • Propranolol • Laxatives • Calcium supplements • Lovastatin	• Bile acid sequestrants • Activated charcoal • Anion exchange resins • Phenytoin • Phenobarbital • Carbamazepine • Rifampin • Amiodarone • Estrogen therapy
	Dietary interference	• Walnuts • Soybean	• Prunes • Herbal remedies
	Others	• Congestive heart failure	• Pregnancy

- Obesity may cause overestimation of absorption.
- This test is not a well-established test in clinical practice. The value of this test should be weighed against risks and cost in each individual patient.

11. Water Deprivation Test (WDT)

INDICATIONS

- To identify the cause of polyuria.
- To differentiate between central diabetes insipidus (DI), nephrogenic DI, and primary polydipsia (PP).

Interpretation

Condition/response	Results
Normal*	• Maximal urine osmolality >800 mOsm/kg when serum osmolality >295 mOsm/kg and sodium >145 mmol/L
Cranial DI	• Urine osmolality <300 mOsm/kg when serum osmolality >295 mOsm/kg and sodium >145 mmol/L • In response to DDAVP: urine osmolality increases to >50%
Nephrogenic DI	• Urine osmolality <300 mOsm/kg when serum osmolality >295 mOsm/kg and sodium >145 mmol/L • In response to DDAVP: urine osmolality increases <10%**
Partial DI (cranial or nephrogenic)	• Urine osmolality 300-800 mOsm/kg when serum osmolality >295 mOsm/kg and sodium >145 mmol/L • In response to DDAVP: ▸ Urine osmolality >10-50% increase – partial cranial DI ▸ Urine osmolality 10-50% increase- partial nephrogenic DI
Primary polydipsia	• Urine osmolality <300 mOsm/kg with low serum osmolality • With WDT – urine osmolality can increase >800 mOsm/kg with increase in serum osmolality • In response to DDAVP: <10% increase in urine osmolality

**Depending on age and renal impairment, urine osmolality of >600 mOsm/kg can be acceptable according to clinical judgment. There are differences on the accepted maximal urine osmolality level, varying from 700 mOsm/kg to 800 mOsm/kg.*
*** Some authors have reported urine osmolality increase <50 %.*

	Primary polydipsia	Central DI	Nephrogenic DI	Partial central DI	Partial nephrogenic DI
Plasma osmolality (mOsm/kg)	<295	>295	>295	>295	>295
Plasma sodium (mmol/L)	<145	>145	>145	>145	>145
Urine osmolality (mOsm/kg)	>800	<300	<300	300 - 800	300 -800
Baseline copeptin	≥5	<2.6	≥21.4	<5	≥21.4
Post DDAVP urine osmolality rise (%)	<10 %	>50 %	<10 % ** (<50 %)	10 -50 %	10 – 50 %

12. Tests to Assess for Autonomic Neuropathy

- A battery of 5 tests suitable for bedside cardiac autonomic function testing indirectly assess both the sympathetic and parasympathetic nervous systems. These tests provide objective evidence of autonomic neuropathy.

Interpretation

Tests	Normal	Borderline	Abnormal
Valsalva ratio	≥1.21	1.11-1.20	≤1.10
(Max – min) heart rate	≥15	11-14	≤10
R-R interval 30:15 ratio	≥1.04	1.01-1.03	≤1
Postural BP drop	≤10	11-29	≥30
Diastolic BP response to hand grip	≥16	11-15	≤10

Results	Interpretation
All 5 tests are normal	Normal
1 of the 3 heart rate tests is abnormal	Early or Mild
2 or more of the heart rate tests are abnormal	Definite or Moderate
2 or more of the heart rate tests are abnormal and one or both BP tests are abnormal	Severe
Any combination of tests that do not fulfil the above criteria	Atypical or Undetermined

APPENDICES A
Results Forms

12

Results form templates
- for ease of documentation and convenience of interpretation
- To appreciate the dynamics of hormone response to stimulation and suppressions

Appendix 1
Growth Hormone Suppression Test

Growth Hormone Suppression Test

Name: **Patient ID:**

Date:

Time (minutes)	Plasma glucose (mmol/L)	Growth hormone (ng/ml or µg/L)
0		
30		
60		
90		
120		

Appendix 3
Insulin Tolerance Test (ITT)

Insulin Tolerance Test (ITT)

Date:
Name: **Patient ID:**
Age: **Weight (kg):**

Insulin dose:

Start time:

Time (minutes)	CBG (mmol/L)	Hypoglycaemic symptoms	Venous BG (mmol/L)	Cortisol (nmol/L)	GH (µg/L)
0					
*Hypoglycaemia event**					
30					
45					
60					
90					
120					

**Hypoglycaemia may occur anytime within the first 30 minutes, usually within 20-30 minutes of insulin injection. BG, blood glucose; CBG, capillary blood glucose; GH, growth hormone.*

Appendix 7
Inferior Petrosal Sinus Sampling (IPSS)

Inferior Petrosal Sinus Sampling (IPSS)

Name: **Patient ID:**

Date:

Time (minutes)	Site	ACTH	IPS/P ACTH	Prolactin	IPS/P PROLACTIN	Rt:Lt IPS/P ACTH intersinus gradient
0	Right					
	Left					
	Peripheral					
2	Right					
	Left					
	Peripheral					
5	Right					
	Left					
	Peripheral					
10	Right					
	Left					
	Peripheral					

ACTH, adrenocorticotropic hormone; IPS, inferior petrosal sinus; Right/Rt, Right IPS catheter; Left/Lt, Left IPS catheter, Peripheral/P, peripheral line; IPS/P, ratio of Right IPS + Left IPS to Peripheral.

Appendix 10
Adrenal Vein Sampling (AVS)

Adrenal Vein Sampling (AVS)

Name: **Patient ID:**

Date:

Time start:

Site	Aldosterone (pmol/L)	Cortisol (nmol/L)	Cortisol AV/P (SI)	A/C ratio
Left AV1				
Peripheral 1				
Left AV2				
Peripheral 2				
Left AV3				
Peripheral 3				
Right AV1				
Peripheral 1				
Right AV2				
Peripheral 2				
Right AV3				
Peripheral 3				

A/C, aldosterone to cortisol ratio; AV, adrenal vein; AV/P, adrenal vein to peripheral vein ratio; P, peripheral; SI, selectivity index.

APPENDIX B
List of Tests and Hospitals Offering Them

No.	Test Name	Referral Centre	Specimen Type	Container	Note
1	17-Hydroxy Progesterone (17OHP)	**Hospital Putrajaya**	Serum	Plain tube	-
2	24-hr urine Metanephrines	**Hospital Kuala Lumpur** **Hospital Putrajaya**	Urine 24-hour	Refer to the respective labs regarding use of preservative	Need endocrinologist/ chemical pathologist signature
3	5-Hydroxy-Indol-Acetic Acid (5 HIAA) 24-hour urine	**Institute of Medical Research**	Urine	24-hour urine bottle with 10 ml 25% HCl	-
4	Adrenocorticotrophic Hormone (ACTH)	**Hospital Kuala Lumpur**	Plasma	EDTA tube	Separate the plasma into another tube
5	Aldosterone	**Hospital Putrajaya**	Plasma	EDTA tube	Separate the plasma into another tube/ frozen
6	Anti-Thyroid Stimulating Hormone Receptor Antibody	**Hospital Kuala Lumpur**	Serum	Plain tube	*State level* - Requires an Endocrinologist's signature *District level* - Requires a General physician's signature *Health clinic (Klinik Kesihatan) level* - Requires a Family Medicine Specialist's signature
7	C-Peptide	**Hospital Kuala Lumpur**	Serum	Plain tube	-
8	Dehydroepiandrosterone Sulphate (DHEAS)	**Hospital Kuala Lumpur**	Serum	Plain tube	-
9	Fructosamine	**Hospital Ampang**	Serum	Plain tube	-
10	Growth Hormone (Somatotrophin)	**Hospital Putrajaya**	Serum	Plain tube	Separate the serum into another tube

No.	Test Name	Referral Centre	Specimen Type	Container	Note
11	Insulin	**Hospital Kuala Lumpur**	Serum	Plain tube	-
12	Insulin-like Growth Factor 1 (IGF-1)	**Hospital Putrajaya**	Serum	Plain tube	Separate the serum into another tube
13	Lithium	**Hospital Bahagia Ulu Kinta** **Hospital Kuala Lumpur**	Blood	Plain tube	-
14	Late Night Salivary Cortisol	**Hospital Pulau Pinang**	Saliva	Salivette kit	Follow instruction manual
15	Noonan Sydrome (PTPN11 Sequence Analysis)	**Institute of Medical Research**	Blood	EDTA tube (2 tubes)	-
16	Panel for Diabetic Antibodies- Anti-islet cells (ICA) , Anti-Glutamic Acid Decarboxylase (GAD),Anti-Insulinoma-Associated Antigen 2 (IA2)	**Institute of Medical Research**	Serum	Plain tube	Separate the serum into another tube
17	Prader-Willi Syndrome (*SNRPN*) MS-PCR	**Institute of Medical Research**	Blood	EDTA tube (2 tubes)	Requires an appointment before sending the sample
18	Renin	**Hospital Putrajaya**	Plasma	EDTA tube	Separate the plasma into other tube/frozen
19	Thyroglobulin	**Hospital Pulau Pinang**	Serum	Plain tube	Separate the serum into another tube/ Requires a specialist's signature

No.	Test Name	Referral Centre	Specimen Type	Container	Note
20	Thyroglobulin Antibody	**Hospital Pulau Pinang**	Serum	Plain tube	Separate the serum into another tube/ Requires a specialist's signature
21	Thyroid Microsomal Antibody/ Thyroid Peroxidase Antibody	**Hospital Pulau Pinang**	Serum	Plain tube	Separate the serum into another tube/ Requires a specialist's signature
22	Vitamin D, Total (25 (OH) Vitamin D)	**Hospital Putrajaya**	Serum	Plain tube	Separate the serum into another tube/ Requires a specialist's signature

References

1965 – 2024

FINALLY.....

We hope this would not end here. Hope a new generation of endo will take up the cudgel and revise the manual in tandem with the latest development in the science of endocrinology
Room for improvement and improvisation

THANK YOU