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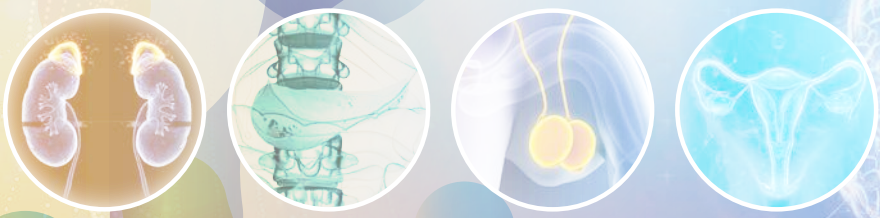
PRACTICAL GUIDE TO

ENDOCRINE DYNAMIC TESTS

KKM Endocrine Subspecialty Service



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The Practical Guide to Endocrine Dynamic Tests is available at https://mems.my/.

The Results Forms in Appendix A serve as templates and can be copied for use in the relevant departments for management of patients.

FOREWORD

This practical guide has been developed with great effort and detail by a group of senior endocrinologists in the Ministry of Health to provide a practical set of evidence-based protocols for commonly used endocrine dynamic tests. It is a timely endeavour that incorporates the best practices from established endocrine centres around the world while adapting to local practice and availability of resources.

The Practical Guide to Endocrine Dynamic Tests will most definitely provide clear guidance to house officers, medical officers, specialists and their nurse assistants, who are required to perform and interpret these endocrine dynamic tests in their day-to-day practice. This is a step forward to enhance the quality of care of our patients with endocrine disease.

With the advancements of new assays and diagnostic tests, this guide will undergo periodic reviews to remain current.

Datuk Dr Zanariah Hussein
Senior Consultant Endocrinologist and Head,
Endocrine Medical Unit, Institut Endokrin Hospital Putrajaya

CONTRIBUTORS

EDITORS

Dr Ijaz Hallaj Rahmatullah
Consultant Endocrinologist,
Hospital Raja Permaisuri Bainun, Ipoh

Dr Subashini Rajoo
Consultant Endocrinologist,
Hospital Kuala Lumpur

Datuk Dr Zanariah Hussein
Senior Consultant Endocrinologist,
Hospital Putrajaya

CONTRIBUTING AUTHORS
CONSULTANT ENDOCRINOLOGISTS

Dr Foo Siew Hui
Hospital Selayang

Datuk Dr Mohamed Badrulnizam Long Bidin
Prince Court Medical Centre

Dr Noor Lita Adam
Hospital Tuanku Jaafar, Seremban

Dr Nor Afidah Karim
Hospital Tuanku Jaafar, Seremban

Dr Nor Shaffinaz Yusoff Azmi Merican
Hospital Sultanah Bahiyah, Alor Setar

Dr Miza Hiryanti Zakaria
Hospital Sultanah Maliha, Langkawi

Dr Norhaliza Mohd Ali
Hospital Sultanah Aminah, Johor Baru

Dr Norhayati Yahaya
Hospital Raja Perempuan Zainab II, Kota Bharu

Dr Shanty Velaiutham
Hospital Pulau Pinang

Dr Ijaz binti Hallaj Rahmatullah
Hospital Raja Permaisuri Bainun, Ipoh

Dr Subashini Rajoo
Hospital Kuala Lumpur

EXTERNAL REVIEWERS

Professor Dr Norlela Sukor
Consultant Endocrinologist,
Hospital Canselor Tunku Muhriz, Cheras

Dr Florence Tan Hui Seng
Consultant Endocrinologist,
Hospital Umum Sarawak, Kuching

Dr Brian Cheong Mun Keong
Consultant Physician,
Hospital Raja Permaisuri Bainun, Ipoh

Dr Siti Sharina binti Anas
Consultant Chemical Pathologist,
Hospital Putrajaya

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INTRODUCTION

In the field of endocrinology, a complete evaluation of hormonal disorders will essentially require a thorough clinical assessment followed by a carefully planned sequence of laboratory investigations to accurately measure specific hormones, peptides or metabolic markers. This enables the attending physician to definitively confirm or exclude a provisional diagnosis of endocrine disease. Following diagnosis, most endocrine diseases may require further intermittent hormonal testing to characterise the state of disease control or response to treatment interventions.

Generally, endocrine disorders may present with hormonal dysfunction, both deficiencies and excessive secretion, varying in severity and correlating to the clinical presentation. In some endocrine disorders, hormonal function may be preserved or minimally deranged. The approach to endocrine testing requires a clear understanding of the physiology involved in the different hormonal axes and the feedback mechanisms involving both trophic and target hormones.

Besides simply measuring the specific hormones in their basal state, additional evaluation with dynamic testing is useful to determine if the elevated hormone levels are suppressible and conversely, whether low hormone levels encountered may respond to stimulation to indicate the underlying hormone reserve. These tests involve the administration of specific exogenous agents and subsequently, interval measurements of the hormone or metabolites concerned. Certain tests require technically demanding invasive hormone sampling by an experienced interventional radiologist and careful decision-making by the endocrinologist is necessary to ensure the tests are well indicated.

There are a multitude of specific provocative or stimulation tests, as well as, inhibitory or suppression tests that have been developed and have evolved over time. Each of these tests required extensive validation and comparative studies before being adopted for standard clinical use, according to evidence-based recommendations. However, due to the inaccessibility of certain stimulatory or inhibitory agents, certain dynamic tests that have been recommended in international guidelines cannot be done locally.

To ensure that dynamic tests are performed optimally, there should be clear understanding and adherence to proper patient preparation and standardised testing protocols with individualised considerations. Close coordination between the clinicians and the respective endocrine laboratory service is a prerequisite to ensuring the samples collected for testing are handled and processed appropriately and efficiently, enabling good accuracy of the test results. A structured format to report the results of dynamic testing would certainly enable clinicians to interpret the tests easily and allow best applicability to case management.

1 GROWTH HORMONE SUPPRESSION TEST FOR ACROMEGALY

According to the 14th Acromegaly Consensus, a 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. For equivocal results, IGF-1 measurements can be repeated, and a growth hormone (GH) suppression test with an oral glucose tolerance test (OGTT) may be beneficial.

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.

Specimen bottles

Investigation	Specimen bottle type
IGF-1	Plain tube *Yellow top with gel vacuum tube*
GH	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Fluoride oxalate containing tube *Grey top bottle*

Preparation

- Cease oral estrogen therapy 4 weeks before the OGTT.
- Fast the patient overnight for at least 8 hours.

Method

1. At 0 minute, collect fasting plasma glucose (FPG) and GH samples using an indwelling branula placed in the cubital fossa.
2. Keep the branula in place.
3. Dilute 75 g of oral glucose in 250 ml of water. Have the patient drink it within 10 minutes.
4. Patients should remain seated or at rest during the test due to the risk of vomiting or vasovagal symptoms when ingesting the glucose drink.
5. Draw blood for plasma glucose and GH at 30, 60, 90 and 120 minutes.

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

Interpretation

- Failure to suppress GH to <1 ng/ml (or <1 µg/L) within 2 hours after glucose ingestion is diagnostic of acromegaly.
- Depending on the sensitivity and accuracy of the GH assay used, some authorities advocate using a cut-off of 0.4 µg/L during OGTT to diagnose acromegaly.
- Body mass index (BMI)-based GH nadir cut-offs can be considered for diagnosing acromegaly: <0.4 µg/L for BMI <25 kg/m^2 and <0.2 µg/L for BMI ≥25 kg/m^2, although, this may be assay dependent.
- **False positives** may occur in patients with anorexia nervosa or other causes of chronic starvation, uncontrolled diabetes mellitus, and liver or renal failure.

2 SHORT SYNACTHEN® TEST (SST)

2.1 Standard dose (250 µg) SST

(also known as corticotrophin stimulation test, adrenocorticotropic hormone (ACTH) stimulation test or cosyntrophin test)

INDICATIONS

- Gold standard to diagnose primary adrenal insufficiency (AI).
- An alternative test for insulin tolerance test for diagnosing secondary hypoadrenalism due to hypothalamic-pituitary disease.
- For the assessment of adrenal function after a prolonged course of corticosteroid/exogenous steroids.
- For the diagnosis of congenital adrenal hyperplasia (CAH).

Preparation

- Medications that should be stopped/omitted before the test.

Medication	When to stop/omit
Estrogen	• 6 weeks before the test, estrogen containing medications should be stopped.
Hydrocortisone	• The last dose of hydrocortisone should be given/administered at least 18-24 hours before the test. ▸ Day before the test, the afternoon dose of hydrocortisone should be omitted. ▸ On the day of the test, the morning dose of hydrocortisone should be omitted.

- Patient does not need to be fasted.

Specimen bottle

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*

Method

- The test can be done at any time of the day in a non-fasted patient.
- Draw a blood sample for cortisol at 0 minute.
- Inject intravenous (IV) or intramuscular (IM) synthetic ACTH, tetracosactrin (Synacthen®) 250 µg and note the time.
- Draw a blood sample for cortisol 30 minutes after the Synacthen® injection.
- Draw a blood sample for cortisol 60 minutes after the Synacthen® injection.

Time (minutes)	Cortisol (nmol/L)
0	
30	
60	

Interpretation

- In a normal response, serum cortisol should rise to ≥500 nmol/L at 30 or 60 minutes after the injection.
- Serum cortisol levels <500 nmol/L at 30 or 60 minutes indicate AI. This cut-off value is assay dependent. The lower reference limits for the 30-minute cortisol response for 5 widely used immunoassays are:

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

2.2 Low dose (1 µg) SST

INDICATIONS

- For the diagnosis of early hypothalamic pituitary disease, as low dose SST provides a higher sensitivity for diagnosing secondary AI compared to the standard dose test.
- When cost is a factor or Synacthen® is not widely available.

Preparation

- Medications that should be stopped/omitted before the test.

Medication	When to stop/omit
Estrogen	• 6 weeks before the test, estrogen containing medications should be stopped.
Hydrocortisone	• The last dose of hydrocortisone should be given/administered at least 18-24 hours before the test. ▸ Day before the test, the afternoon dose of hydrocortisone should be omitted. ▸ On the day of the test, the morning dose of hydrocortisone should be omitted.

- The patient does not need to be fasted.

Specimen bottle

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*

Method

1. The test can be done at any time of the day in a non-fasted patient.
2. Draw a blood sample for cortisol at 0 minute.
3. Inject IV or IM Synacthen® 1 µg and note the time.
4. Draw a blood sample for cortisol 30 minutes after the Synacthen® injection.

Interpretation

- A normal response results in peak serum cortisol ≥500 nmol/L within 30 minutes.

2.3 SST for congenital adrenal hyperplasia in adults

INDICATION

- To confirm the diagnosis of non-classical CAH when the morning serum 17-hydroxyprogesterone (17-OHP) is >6 nmol/L.
  - The screening test for CAH is serum 17-OHP taken in the morning between 7.30-8.00 a.m.
  - In women who have regular menstrual cycles, the sample should be taken during the follicular phase.
  - For women with amenorrhoea or infrequent menstrual cycles, the sample can be drawn on any random day.
  - Interpreting the screening 17-OHP test:

Serum 17-OHP concentration in adult women	Interpretation
>30 nmol/L	Classic CAH
6-30 nmol/L	Proceed to SST or ACTH stimulation test
<6 nmol/L	Unlikely CAH

Specimen bottle

Investigation	Specimen bottle type
Serum 17-OHP	Plain tube *Yellow top with gel vacuum tube*

Method

1. Inject IV Synacthen® 250 µg.
2. Draw a blood sample for serum 17-OHP at 60 minutes.

Interpretation

- Serum 17-OHP >30 nmol/L at 60 minutes post ACTH stimulation test confirms the diagnosis of non-classical CAH.
- Serum 17-OHP <30 nmol/L at 60 minutes post-test excludes CAH.

Caveats

- Patients with conditions like nephrotic syndrome and liver disease, those in the immediate postoperative period, or those requiring intensive care may exhibit lower levels of corticosteroid-binding globulin and albumin, leading to reduced cortisol measurements. The use of estrogen-containing oral contraceptives will result in higher corticosteroid-binding globulin levels, consequently increasing cortisol measurements.
- Standard dose SST may not be the gold standard test for secondary or tertiary AI, particularly with recent onset of the disease, i.e. <3 months.

3 CORTISOL DAY CURVE ON HYDROCORTISONE

Some experts have proposed using a hydrocortisone day curve to determine the adequacy of glucocorticoid replacement. However, the validity of this method for assessing the quality or sufficiency of glucocorticoid replacement remains controversial. Despite this, it may offer useful information for patients suspected of having inadequate glucocorticoid replacement.

INDICATION

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

Preparation

- Stop all estrogen therapy 6 weeks before the test.
- Explain to the patient and remind them not to take their morning dose of hydrocortisone before the test.
- The patient does not need to be fasted.
- Insert and secure an 18G or 20G branula.

Specimen bottle

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*

Methods

Based on the available literature and laboratory reports from established endocrine centres, there are several methods for performing this test. The 2 methods that have been adapted for the purpose of this guide are presented below.

Method 1

1. Obtain sample for serum cortisol at 8 a.m. before the morning dose of hydrocortisone (T0).
2. Give the patient their usual morning dose of hydrocortisone and note the time.

3. Obtain another 7 samples of serum cortisol after the morning dose of hydrocortisone using the following schedule:

Hours after morning dose of hydrocortisone	Sample number	Administering the remaining doses of hydrocortisone	
		Patient on 2 daily doses	Patient on 3 daily doses
1	T1	-	-
2	T2	-	-
5	T5	-	2nd dose after T5
7	T7	2nd dose after T7	-
9	T9	-	3rd dose after T9
10	T10	-	-
11	T11	-	-

Method 2

1. Patient administers their morning dose of hydrocortisone at home and notes the time.
2. The 1st blood sample is taken on arrival at the facility and labelled as x hours/minutes (Tx) post-morning dose.
3. Obtain the remaining blood samples using the following schedule:

Sample time	Sample no.
Before the mid-day dose	2
1 hour after the mid-day dose	3
Before the evening dose	4
1 hour after the evening dose	5

Interpretation

- Aim for adequate cortisol levels throughout the day (peak <900 nmol/L, trough >100 nmol/L).
- As a general guideline, the values below are commonly observed.
  - Morning peak cortisol: 500-800 nmol/L
  - Mid-day peak cortisol: 400-500 nmol/L
  - Post-evening dose cortisol: 300-400 nmol/L
  - Late evening cortisol (6-9 pm): <250 nmol/L
- Minor deviations do not necessarily require dose adjustments, particularly if the patient is well.
- If serum cortisol concentrations before the afternoon and evening doses are low (<100 nmol/L), it indicates under-replacement.

4 ANTERIOR PITUITARY STIMULATION TEST

4.1 Insulin tolerance test (ITT)

INDICATIONS

- Assessment of adrenocorticotropic hormone (ACTH) and cortisol reserve.
- Assessment of growth hormone (GH) reserve in patients with pituitary or hypothalamic disease.
- Assessment of GH reserve in children with definite growth retardation.
- The test may be combined with serum gonadotropin releasing hormone (GnRH) or thyrotrophin releasing hormone (TRH) tests although this is rarely indicated.

Contraindications

Absolute contraindications

- Ischaemic heart disease.
- Epilepsy.
- Unexplained syncope.
- Untreated hypothyroidism (impairs GH and cortisol response).
- Severe long standing hypoadrenalism – liver glycogen stores are depleted causing severe hypoglycaemia during ITT or morning serum cortisol is < 100 nmol/L.
- Glycogen storage disease.

Relative contraindication

- Age >65 years old.

Side-effects

- Sweating, palpitations, loss of consciousness and rarely convulsion due to severe hypoglycaemia.

4. Anterior Pituitary Stimulation Test

Preparation

- Pre-test electrocardiogram (ECG) should be done, and must be normal.
- Peri-pubertal children (bone age >10 years) must be primed.

Peri-pubertal children	Pre-test priming medications
Male	3 days before the test: Single 100 mg intramuscular (IM) testosterone enanthate
Female	3 days before the test: Start daily 100 µg oral ethinylestradiol or 0.625 mg oral conjugate estrogen x 3 days

- Hydrocortisone should be discontinued 24 hours before the test.
- The patient should fast overnight (water is permitted).
- Medications can be withheld and given after completion of the test, i.e., by lunch time.

Materials required

- 50 ml of 50% Dextrose must be available for immediate administration if there is persistent severe hypoglycaemia during the test.
- Biscuits and juice.
- Glucometer and test strips.
- An indwelling branula – 18G to 20G.
- A short- acting insulin (e.g. actrapid).
- A doctor present during the insulin administration until the resolution of hypoglycaemia.

Specimen bottles

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*
GH	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Fluoride oxalate containing tube *Grey top bottle*

Method

1. Weigh the patient and calculate the short-acting insulin dose according to the patient's body weight.
   a. The usual dose is 0.15 U/kg body weight. For hypopituitary patients, start with 0.1 U/kg.
   b. Patients with diabetes, acromegaly and Cushing's syndrome use 0.2-0.3 U/kg.
2. The patient should be in supine position throughout the test.
3. Insert the 18G or 20G branula into the antecubital fossa with good access for blood taking and for administration of glucose, if necessary.
4. Obtain the baseline venous blood at 0 minute for plasma glucose, GH and cortisol investigations (4 mls/tube).
5. Administer the calculated dose of the short-acting insulin intravenously, in a single push, followed by a 10 ml saline flush.
6. Note the time.
7. Monitor the capillary blood glucose (CBG) every 15 minutes using the glucometer and test strips, and for symptoms of hypoglycaemia.
   a. If the patient complains of hypoglycaemic symptoms such as fatigue, sweating, hunger, palpitations or dizziness, monitor the CBG more frequently.
8. The glucose level will drop to the lowest level within 20-30 minutes of the IV short-acting insulin, followed by spontaneous resolution.
   a. Adequate hypoglycaemia, i.e. plasma glucose <2.2 mmol/L, should be symptomatic.
   b. Record the symptoms in the patient's notes.
9. If, by 45 minutes, adequate hypoglycaemia is not reached, and patient is asymptomatic of hypoglycaemia, repeat the IV short-acting insulin at a higher dose , e.g. 50% higher than the initial dose, take the same blood samples and start the time again from 0 minute.
10. Once hypoglycaemia is achieved, obtain samples for plasma glucose, GH and cortisol.
11. Once the hypoglycaemic blood samples are obtained, the patients do not need to remain in hypoglycaemia.
12. Reverse the hypoglycaemia with oral treatment (biscuits or juice). However, if the hypoglycaemic symptoms are severe, consider giving 10-20 ml of IV Dextrose 20% to 50% or 1 mg (1 ampoule) of IM glucagon, and continue sampling.
    a. Obtain samples for plasma glucose, GH and cortisol at 30, 45, 60, 90 and 120 minutes.
    b. Flush the branula with saline between the sampling.
13. If the patient has an acute adrenal crisis, administer 0.9% saline and IV 100mg hydrocortisone.
14. The doctor should remain with the patient from the time of the first insulin administration until hypoglycaemia occurs. They can leave once the glucose level starts to rise following the hypoglycaemia.
15. Once the test is completed, the patient should be given a supervised meal.
16. The patient should not drive for 2 hours after the test.

Example of documenting the ITT

Patient's name:	Patient's ID:
Date:	Initial insulin dose:
Start time:	Weight:

Time (minutes)	CBG (mmol/L)	Hypoglycaemic symptoms	Plasma blood glucose (mmol/L)	Cortisol (nmol/L)	GH (µg/L)	Notes
0						
Hypoglycaemic event						
30						
45						
60						
90						
120						

Hypoglycaemia may occur anytime within the first 30 minutes, and usually within 20-30 minutes of the IV short-acting insulin.

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.

4.2 Glucagon stimulation test (GST)

INDICATION

- To assess for GH deficiency, particularly when insulin-induced hypoglycaemia is contraindicated.

Contraindications

- Pheochromocytoma or insulinoma.
- Severe hypocortisolism, i.e. when 9 a.m. cortisol level is <100 nmol/L)
- Malnourished patients or starvation of >48 hours.

Side effects

- Though nausea is common (30% of patients), patients rarely vomit. For patients with nausea, an IV antiemetic can be considered.
- Late hypoglycaemia can rarely occur, and patients should be advised to eat small and frequent meals after completing the test.

Preparation

- The patient's pretest serum free thyroxine level must be within normal range, as thyroxine deficiency might depress GH response.
  - If the level is low, replace the thyroxine and re-assess.
- The patient's last dose of hydrocortisone should be at midday on the day before the test.
- Omit the evening dose on the day before the test, and the morning dose on the test day.
- Patient must be fasted from midnight.
- Patient should be weighed to calculate the glucagon dose.
- Prepare 8 sample bottles each for plasma glucose and GH.

Specimen bottles

Investigation	Specimen bottle type
GH	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Fluoride oxalate containing tube *Grey top bottle*

4. Anterior Pituitary Stimulation Test

Method

- Weigh the patient to determine the glucagon dose needed for the test.
  - Adult dose: 1 mg or 1.5 mg if body weight is >90 kg.
  - Children dose: 15 µg/kg.
- With the patient resting in supine position, insert a branula into a vein at the antecubital fossa.
- If heparin-saline is injected into the branula, wait for approximately 5-10 minutes before taking the first blood samples.
- Obtain the baseline venous blood at 0 minute for CBG, plasma glucose and GH.
- Repeat the heparin-saline block after the samples are obtained.
- Administer the IM glucagon – the deltoid may be a suitable area.
- Repeat the blood sampling for plasma glucose, GH and CBG at 60, 90, 120, 150, 180, 210 and 240 minutes.
- Observe the patient for 2 hours after the last blood draw.
- The patient can be discharged home if the plasma glucose level is >4 mmol/L.

Example of documenting the GST

Patient's name:		Patient's ID:
Age:		Sex:
Weight:	Height:	BMI:
Glucagon dose:		Diabetes mellitus: Yes/No
Date:		

Time (minutes)	CBG (mmol/L)	Plasma blood glucose (mmol/L)	GH (µg/L)
0			
Inject IM glucagon			
60			
90			
120			
150			
180			
210			
240			

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 and requires more data.

4.3 Gonadotrophin-releasing hormone (GnRH)/Luteinising hormone releasing hormone (LHRH) test

INDICATIONS

- To further investigate possible gonadotrophin deficiency.
- To confirm precocious puberty.

Preparation

- For confirming precocious puberty, do not prime with sex steroids.
- If the GnRH test is being done alone, the patient does not need to fast overnight.
- Requires IV 100 µg LHRH/GnRH (Gonadorelin).

Specimen bottles

Investigation	Specimen bottle type
Follicle-stimulating hormone (FSH)	Plain tube *Yellow top with gel vacuum tube*
Luteinising hormone (LH)	Plain tube *Yellow top with gel vacuum tube*
Oestradiol	Plain tube *Yellow top with gel vacuum tube*

Method

1. Insert an indwelling branula preferably into the antecubital fossa.
2. Obtain baseline blood samples for LH, FSH, and testosterone for males or oestradiol for females.
3. Inject IV Gonadorelin 100 µg and flush the branula with saline.
4. Repeat sampling for LH and FSH only, at 30 and 60 minutes after the IV Gonadorelin.

Interpretation

- Normal peaks can occur at either 30 or 60 minutes.
  - LH should exceed 10 U/L
  - FSH should exceed 2 U/L
- An inadequate response may be an early indication of hypopituitarism.
- Gonadotrophin deficiency is diagnosed with baseline hormone level rather than the dynamic response.
  - In males: Low testosterone in the absence of raised baseline gonadotrophins.
  - In females: Low oestradiol without elevated baseline gonadotrophins and no response to clomiphene.
- Pre-pubertal children should not have any LH and FSH response to LHRH.
  - Response to LHRH indicates sex steroids are present, i.e. secondary to precocious puberty; the pituitary is "primed" and will respond to the LHRH.

4.4 Thyrotrophin releasing hormone (TRH) stimulation test

INDICATIONS

- To assess thyroid stimulating hormone (TSH) reserve.
- To differentiate between pituitary and hypothalamic causes of TSH deficiency.
- To diagnose thyroid hormone resistance.
- To diagnose TSH-secreting pituitary tumour (thyrotophinoma, TSH-oma)

Precautions

- Patients should be warned about the potential transient side effects after the TRH injection such as, metallic taste in the mouth, flushing, mild nausea and the desire to micturate.
- TRH could increase the blood pressure significantly.
- TRH injections may cause pituitary tumour haemorrhage with severe headache. However, it rarely occurs.
- In pituitary macroadenoma, TRH could precipitate pituitary apoplexy.
- In patients on thyroxine treatment, TRH stimulation test is rarely done as it requires thyroxine treatment to be withheld for 3 weeks before the test.

Contraindication

- Known allergies.

Preparation

- Overnight fasting is not necessary.
- IV TRH 200 µg.

Specimen bottles

Investigation	Specimen bottle type
TSH	Plain tube *Yellow top with gel vacuum tube*
FT4	Plain tube *Yellow top with gel vacuum tube*

Method

1. Patient should be lying in a supine position.
2. Insert an indwelling branula.
3. Obtain baseline (0 minute) blood samples for TSH and free thyroxine (FT4).
4. At 9 a.m. inject TRH 200 µg via slow IV injection over 2 minutes, then flush the branula with saline.
5. Repeat blood sampling for TSH at 30 and 60 minutes after the injection

	0 minute	30 minutes	60 minutes
TSH (mIU/L)			
FT4 (pmol/L)		-	-

Interpretation

- Normal results: TSH rises to >5 mIU/L with its 30 minute value exceeding the 60-minute value.
- If the 60-minute value > 30-minute value: Usually indicates primary hypothalamic disease but may sometimes be seen in pituitary disease or primary hypothyroidism.
- TSH fails to respond to TRH: Usually indicates primary pituitary disease. The TSH remains suppressed in hyperthyroidism and euthyroid Grave's ophthalmopathy.
- Exaggerated TSH response: Hypothyroidism will elicit an exaggerated TSH response. However, with the currently available TSH assays, baseline levels are adequate to determine hypothyroidism and dynamic testing is not usually needed.
- Thyroid hormone resistance is associated with a normal or exaggerate response, while TSH-oma elicits no response to TRH.

4. Anterior Pituitary Stimulation Test

TSH response (mIU/L) after TRH stimulation	
Normal	>5 mIU/L 30-minute > 60-minute
1° hypothalamic disease	60-minute > 30-minute
1° pituitary disease	No response
Hyperthyroidism	TSH remains suppressed
Thyroid hormone resistance	Normal or exaggerated response
TSH-oma	No response

5 TESTS FOR CUSHING'S SYNDROME

5.1 Approach to suspected endogenous Cushing's syndrome

See Figure 5-1 (page 35).

1. Exclude Cushing's syndrome due to exogenous steroids.
2. Conduct the following screening/diagnostic tests:
   a. Dexamethasone suppression tests – Overnight dexamethasone suppression test (ODST) or the low-dose dexamethasone suppression test (LDDST).
   b. 24-hour urinary cortisol – at least 2 tests.
   c. Late night salivary test – at least 2 tests.
3. A diagnosis of Cushing's syndrome is confirmed if any 2 of the above screening tests are positive.
4. Preferred screening tests in specific conditions:

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.

5. On confirmation of Cushing's syndrome, perform test for ACTH. Localisation tests will depend on ACTH results, i.e., ACTH-dependent or ACTH-independent.
   a. In ACTH-dependent Cushing's syndrome (ACTH is normal or elevated), proceed with a magnetic resonance imaging (MRI) pituitary, computed tomography scan (CT) thorax or CT abdomen. If the MRI pituitary reveals a pituitary adenoma <6 mm, perform an inferior petrosal sinus sampling.
   b. In ACTH-independent Cushing's syndrome (ACTH low), proceed with a CT adrenal gland.
6. In addition, perform a high-dose dexamethasone suppression test (HDDST) to distinguish between Cushing's disease and ectopic ACTH secretion.

Note: Random serum cortisol is not recommended as a screening test; a normal random serum cortisol level does not exclude Cushing's syndrome.

5.2 Dexamethasone suppression tests

5.2.1 Overnight dexamethasone suppression test (ODST)

Method

1. Prescribe and instruct patients to take oral dexamethasone 1 mg at 11 p.m.-12 a.m., the day before the test.
2. Obtain the serum cortisol the next morning at 8 a.m.-9 a.m.

Interpretation

- Serum cortisol <50 nmol/L indicates normal response. Values above this are suggestive of Cushing's syndrome.

5.2.2 48-hour low dose dexamethasone suppression test (LDDST)

Method

1. The test can be done as an outpatient procedure with written instructions.
2. 0.5 mg oral dexamethasone is prescribed every 6-hours for 48 hours from 12 p.m. on the 1st day; the last dose is at 6 a.m. on day-2.
3. Obtain sample for serum cortisol on day-3 at 8 a.m.-9 a.m.

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.

Interpretation

- Serum cortisol <50 nmol/L indicates normal response. Values above this are suggestive of Cushing's syndrome.

5.2.3 High dose dexamethasone suppression test (HDDST)

See Section 5.6.

5.3 24-hour urinary cortisol

Method

1. Instruct the patient on how to complete a 24-hour urine collection:
   a. The collection begins with an empty bladder.
   b. Patient is not to drink an excessive amount of fluid during the collection period.
2. Patient is to repeat a second 24-hour urine collection on another day.

Interpretation

- >3-times elevation from the upper limit of normal is suggestive of Cushing's syndrome.

5.4 Late night salivary cortisol

Preparation

- Instruct the patient to not brush or floss their teeth before collecting the sample.
- Instruct the patient to not eat or drink for 15 minutes before collecting the sample.
- The patient must not use any steroid inhalers for 24 hours before the saliva sample collection.
- The patient must not use any creams or lotions that contain steroids such as hydrocortisone cream, bethamethasone cream, etc.

- The patient should not collect saliva sample if they have any bleeding from the oral cavity.
- Prepare the supplies required:
  - Saliva Collection Kit containing SARSTEDT Salivette sample tube.
  - Minimum 2 samples will be required on 2 separate days.

Method

1. Provide the patient with a Saliva Collection Kit (Salivette) containing the Cortisol-Saliva Collection instructions and ask them to follow it.
2. Instruct the patient to collect the specimen (specimen volume required is 1.5 ml) between 11 p.m. and 12 midnight, and record the collection time in the Cortisol-Saliva Collection instruction sheet.
3. Instruct the patient to return the Cortisol-Saliva instruction sheet with the appropriately labelled Salivette to the laboratory.
4. Instruct the patient to repeat a 2nd sample collection the following day.

Interpretation

- The normal range is <11.3 nmol/L. Values above this is suggestive of Cushing's syndrome.

5.5 ACTH measurement

Method

- ACTH is measured once the diagnosis of Cushing's syndrome is confirmed.
- It should be taken together with serum cortisol at 8 a.m.-9 a.m.
- The blood sample for ACTH should be kept in an ice water bath and sent to the laboratory.
- Centrifugation and freezing should be done as soon as possible to avoid degradation and a falsely low result.

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.

5.6 High-dose dexamethasone suppression test (HDDST)

Specimen bottles

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*
ACTH	EDTA tube *Purple top with gel vacuum tube*

Method

- The test can be done in the outpatient setting with written instructions.
- Serum cortisol is obtained at baseline (Day 1 at 8 a.m.)
- Oral dexamethasone 2 mg is prescribed to be taken 6 hourly starting at 12 p.m. on Day 1.
- Serum cortisol is repeated after the test on Day 3 at 8 a.m.

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.	-	✔	✔	-

Interpretation

- A decrease of serum cortisol by >50% from baseline is suggestive of pituitary Cushing's disease.
- However, 10% of patients with ectopic ACTH secretion have suppressed cortisol with high doses of dexamethasone.
- Some patients with pituitary tumours can fail to suppress serum cortisol.
- Sensitivity and specificity of this test are 81% an 79%, respectively.

5.7 Inferior petrosal sinus sampling (IPSS)

INDICATION

- To differentiate between ectopic ACTH secretion (EAS) and Cushing's disease in patients with ACTH-dependent Cushing's syndrome especially when the source of ACTH is not definite.

Contraindications

- Known allergy to contrast dye.
- Ischaemic heart disease.
- Known orthopnoea.
- Known bleeding tendencies.

Preparation

- Metyrapone and ketoconazole need to be stopped 1 week before IPSS, as the test should be carried out when cortisol levels are elevated.
- The pathologist and laboratory technician should be informed of the test a few days in advance and on the day of the procedure.
- Order Desmopressin (DDAVP) 10 µg or synthetic human CRH (Corticorelin) 100 µg, if available, in advance from the pharmacy.
- One day before the test:
  - Prepare and correctly label the specimen bottles.
  - Prepare the syringes for sampling and flushing the branula.
  - Arrange for ice to be available on the day of the procedure.
  - Check for the patient's full blood count (FBC), renal profile (RP), and prothrombin time (PT)/INR.
  - Take the patient's consent after informing of the risks of the procedure such as bleeding tendencies, cerebrovascular accidents (CVA), dye allergy and pulmonary embolus.
- Fast patient overnight or at least 4 hours before the procedure.
- On the day of the procedure, measure the serum cortisol level (8 a.m. sample) and only proceed if the serum cortisol is >275 nmol/L (10 µg/dl).
- During the procedure:
  - There should be 2-3 people to assist in the sample taking and handling.
  - The ice bath should be prepared to keep and transport the ACTH samples.

Specimen bottles

Investigation	Specimen bottle type
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*
ACTH	EDTA tube *Purple top with gel vacuum tube*
Prolactin	Plain tube *Yellow top with gel vacuum tube*

Method

1. The procedure is performed by an interventional radiologist.
2. Under aseptic technique, 2 catheters are placed into the femoral veins at the level of the groin.
3. Following this, with fluoroscopic guidance, the catheters are navigated to the right (R) and left (L) inferior petrosal sinus (IPS), which drain the pituitary gland.
4. A 3rd catheter is placed peripherally in the arm or the femoral sheath (P)
5. Baseline samples for ACTH and prolactin are taken at 0 minute; the ACTH sample should be immediately placed in the ice bath.
6. A bolus IV Desmopressin or IV CRH, if available, is injected over 1 minute through P.
   a. Dose of IV Desmopressin: 10 µg.
   b. Dose of IV CRH in adults: 100 µg
   c. Dose of IV CRH in children: 60 µg/m^2
7. Samples from the 3 sampling sites (L, R and P) should be taken simultaneously at 5 and 10 minutes after the injection.
   a. At some centres, an additional sample is taken at 2 or 3 minutes after the injection.
8. The ACTH samples should be kept in the ice bath immediately after it is obtained, and all samples (0, 5 and 10 minutes or 0, 2/3, 5 and 10 minutes) should be taken directly to the laboratory for processing.

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.

9. Monitor the patient post-procedure and look for complications.
   a. When the procedure is performed by an experienced radiologist, the incidence of serious complications such as CVA is 0.2%.
   b. Brain stem infarction and 1 case of pulmonary embolism have been reported.

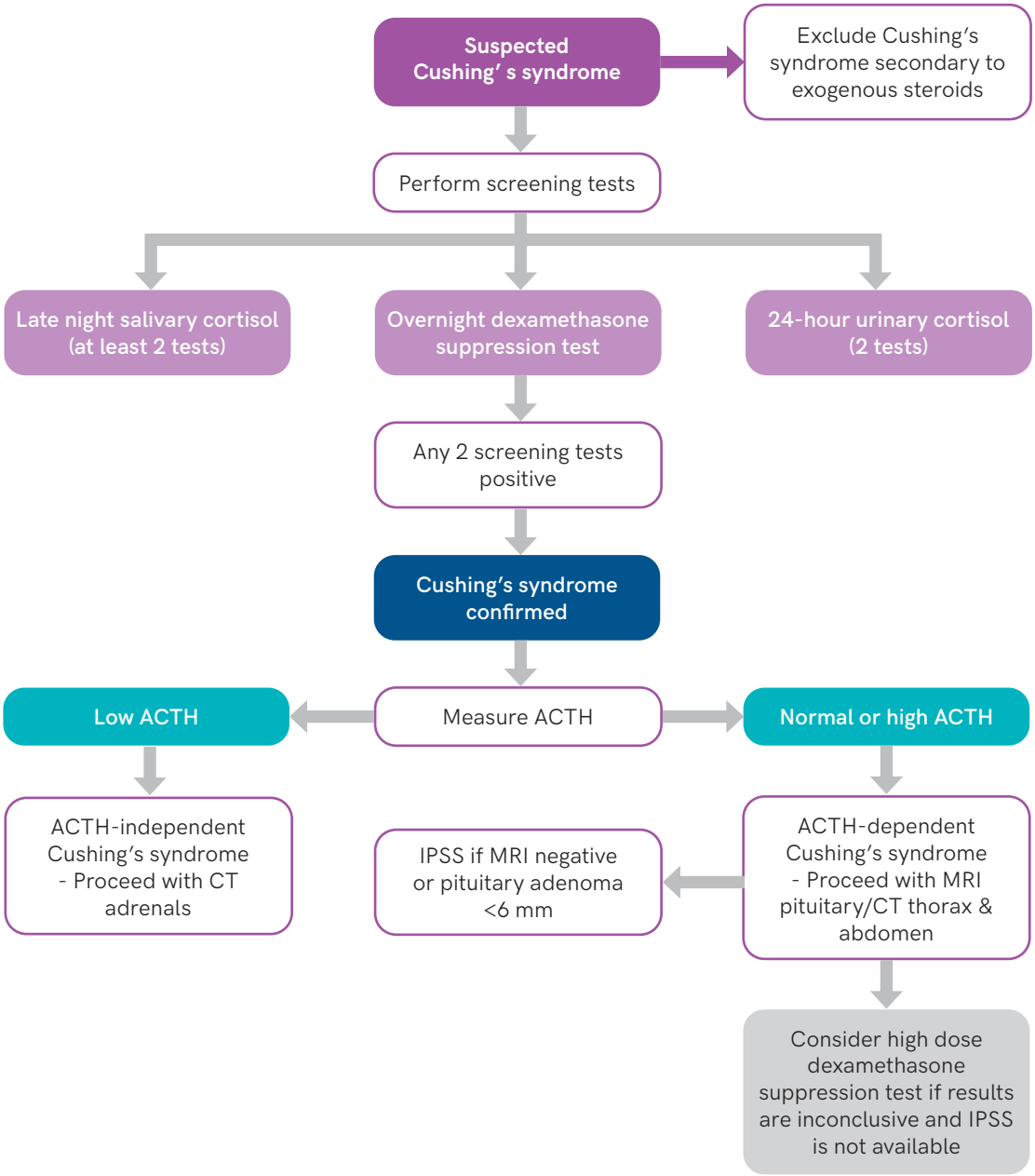
Interpretation

- IPS/P ACTH ratio of ≥2.0 before DDAVP or CRH administration or ≥3.0 after the injection suggests a central (pituitary) lesion with sensitivity and specificity of 95% and 100%, and 100% sensitivity, respectively).
- IPS/P prolactin of ≥1.8 suggests successful catheterisation during IPSS.
- IPS/P prolactin of <1.3 indicates unsuccessful cannulation or abnormal pituitary efflux or aspiration of samples from an aberrant collateral vessel.
- When the IPS/P prolactin is <1.3, the prolactin-adjusted IPS/P ACTH can improve the differentiation between Cushing's disease and EAS, which can reduce false negative results.
- To calculate the prolactin-adjusted IPS/P ACTH ratio – divide peak post-DDVAP IPS/P ACTH with the ipsilateral baseline IPS/P prolactin.
  - ≤0.8: Suggests EAS
  - ≥1.3: Indicates Cushing's disease
  - 0.8-1.3: Intermediate results.
- Perform lateralisation by measuring the intersinus gradient, i.e. the ratio between left and right IPS/P ACTH – Divide the higher IPS/P ACTH with the lower IPS/P ACTH.
  - A ratio of ≥1.4 predicts lateralisation of a microadenoma at the site with the higher IPS/P ACTH.

Caveats

- IPSS has 100% sensitivity after CRH injection. DDAVP elicits a response similar to CRH, and is an alternative to CRH. However, CRH is currently unavailable globally.
- In cases where the IPS/P ACTH gradient is not consistent with a pituitary source, peripheral ACTH response (>35%) to CRH/DDAVP administration suggests a central aetiology rather than an ectopic source.
- In the absence of an appropriate bilateral IPS catheterisation, a lack of significant IPS/P ACTH gradient does not rule out a pituitary source as the underlying aetiology for Cushing's syndrome.
  - In addition, confirmation of accurate venous sampling in only 1 IPS may not rule out a pituitary source in the contralateral side of the pituitary gland due to variable venous drainage.
- Petrosal sinus sampling is of limited value in distinguishing between patients with Cushing's syndrome and normal individuals or those with pseudo-Cushing's states.
  - Therefore, a diagnosis of ACTH-dependent Cushing's syndrome should be established before referring a patient for IPSS.

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



ACTH, adrenocorticotropic hormone; CT, computed tomography; IPSS, inferior petrosal sinus sampling; MRI, magnetic resonant imaging.
Adapted from Maria D, et al. Lancet Diabetes Endocrinol, 2021.

6 TESTS FOR FEMALE INFERTILITY

6.1 Screening for ovulation

Background

- Luteinising hormone (LH) and follicle stimulating hormone (FSH) rise for approximately 48 hours ("surge") at the onset of the ovulatory phase of the menstrual cycle.
- Progesterone production rises in the ovulatory phase to a maximum during the luteal phase.
- Baseline body temperature rises by >0.5°C during the ovulatory phase peaking about 8 days after the LH surge.

INDICATION

- To confirm ovulation in a woman **with menstruation** presenting with infertility.

Specimen bottle

Investigation	Specimen bottle type
Serum progesterone	Plain tube *Yellow top with gel vacuum tube*

Method

1. Obtain blood sample for progesterone in the mid-luteal phase, i.e., on days 18, 21 and 24 of the menstrual cycle for at least 2 cycles.
2. A more intensive screening regimen is undertaken in the in-vitro fertilisation (IVF) clinic, and a referral is an alternative option.

Note: During a 28-day cycle the mid-luteal phase would be on day 21. During a 35-day cycle this would be on day 28 (i.e., 7 days prior to the next predicted menses). In more prolonged cycles, this can be repeated weekly until menses. For women with irregular cycles, 1 approach could be to request testing for progesterone on days 18, 21 and 24. This should be undertaken for at least 2 cycles.

Interpretation

- Progesterone >30 nmol/L between days 18 and 24 indicates an adequate luteal phase (production of progesterone by granulosa cell) and therefore evidence of intact ovulation.

6.2 Progesterone challenge test

Method

1. Prescribe oral medroxyprogesterone 10 mg daily for 5 days to induce uterine bleeding.

Interpretation

- If patient has bleeding within 1 week of stopping progesterone, then she has a sufficient amount of estrogen to stimulate endometrial growth and a normal outflow tract, but lacks progesterone due to anovulation.
- If patient has no bleeding, she either has an outflow-tract defect, or is estrogen-deficient from ovarian failure or dysfunction of the hypothalamic-pituitary axis.
  - If a combination oral contraceptive pill (OCP) for 21 days does not induce bleeding, the patient likely has an outflow tract obstruction.

7 TESTS FOR MALE HYPOGONADISM/INFERTILITY

7.1 Human chorionic gonadotropin (hCG) stimulation test

Human chorionic gonadotropin (hCG) stimulates testicular Leydig cells to secret androgens via the luteinising hormone (LH) receptors.

INDICATIONS

- To identify any intra-abdominal testicular tissue in patients with true bilateral cryptorchidism.
- To differentiate between constitutional pubertal delay and hypogonadotropic hypogonadism.
- To investigate patients with sex differentiation disorder.

Precautions

- The test should not be performed before 2 weeks of age.
- There may be some virilisation effect (increase in testicular size and erection) in boys with normal testes.
- If a gonadotropin releasing hormone (GnRH) test is planned, it should be carried out before or >6 weeks after the hCG test; hCG has a long half-life.
- Headaches and/tiredness are reported side effects of the test.

Preparation

- Serum testosterone (T) can be sent to local laboratories.
- Serum for dihydrotestosterone (DHT) and androstenedione (sometimes abbreviated as A4) will need to be sent to private laboratories as the service is not provided in Ministry of Health (MoH) Malaysia laboratories.
- Samples do not need to be transported in ice.

Specimen bottles

Investigation	Specimen bottle type
Serum T	Plain tube *Yellow top with gel vacuum tube*
Serum DHT	Plain tube *Yellow top with gel vacuum tube*
Serum androstenedione	Plain tube *Yellow top with gel vacuum tube*

Method

hCG stimulation test is performed as a 3-day protocol.

1. **Day 1** – Between 8 a.m.-9 a.m., obtain baseline blood samples for T. In addition, send the sample for androstenedione and DHT if a steroid biosynthetic defect is suspected.
2. **Day 1** – Immediately following baseline sample collection, administer weight-based intramuscular injection (IM) hCG using the following dosing chart:

Weight (kg)	IM hCG dose (IU)
<5	500
5- <10	1000
10-15	1500
>15	3000

3. **Day 4** – Repeat blood sampling for the 3 tests, 72 hours after the hCG injection.

Interpretation

Normal responses are age-based:

- **Infancy** – A normal serum T increment after hCG may vary from 2-fold to 10- up to 20-fold.
- **Childhood** – Serum T increment after hCG is between 5- and 10-fold.
- **Puberty** – As the baseline T concentration is higher, the increment is less, i.e., 2- to 3-fold.
- **6 months-puberty** – Normal T/DHT ratio is <20 before hCG stimulation and <27 after.

Testosterone and androgen response

Condition	Response
Absence of testes	No response
Primary gonadal failure or anorchia	An absent response with an exaggerate LH/FSH response to LHRH stimulation
T biosynthesis defect	Increase in precursor steroid secretion
5 α-reductase deficiency	T/DHT ratio is <20 before hCG stimulation but >27 after
17 β-hydroxysteroid dehydrogenase deficiency	Androstenedione/T ratio is >20 after hCG stimulation

7.2 Tests for monitoring men undergoing spermatogenesis induction

Treatment may take from a few months to up to 2 years. Though many men will not achieve normal sperm counts as defined by the World Health Organization (WHO) standard, low sperm count does not preclude fertility.

	WHO 2010	WHO 2021
Semen volume (ml)	1.5 (1.4-1.7)	1.4 (1.3-1.5)
Total sperm number (10^6 per ejaculate)	39 (33-46)	39 (35-40)
Total motility (%)	40 (38-42)	42 (40-43)
Progressive motility (%)	32 (31-34)	30 (29-31)
Non-progressive motility (%)	1	1 (1-1)
Immotile sperm (%)	22	20 (19-20)
Vitality (%)	58 (55-63)	54 (50-56)
Normal forms (%)	4 (3-4)	4 (3.9-4)

Comparing the differences in parameter values in 2010 and 2021. 5th and 6th editions of the WHO manual for seminal fluid analysis.

Method

Step 1: Initiating hCG

1. Stop T treatment and initiate IM hCG at 2500 U for 3-times/week.

   Note: most protocols initiate hCG at 1500 or 2000 U 3-times/week. Here 2500 U is the initiation dose to avoid wastage as 1 ampoule of hCG contains 5000 U. Some centres initiate hCG 2500 U twice/week or 1500 U 3 times/week as cost saving measures (both regimens use 1 ampoule/week).

2. Every 1-2 months, titrate the dose to achieve serum T 15-30 nmol/L.
3. If the serum T reaches 15-30 nmol/L, maintain the hCG dose and initiate semen analysis every 1-3 months.
4. If the serum T is maintained for 6 months, and sperm count is >5-10 million/ml, continue the same hCG dose and wait for spontaneous or successful assisted reproductive technique (ART) pregnancy.

Step 2: Adding human menopausal gonadotropin (hMG) (follicle stimulating hormone; FSH)

1. If the serum T is maintained at normal levels for 6 months, but the sperm count is still <5 million/ml, add hMG (FSH) 75 U 3-times/week.
2. Perform seminal fluid analysis 1-3 monthly for 6 months.
3. Titrate the hMG dose to up to 150 U 3-times/week if the sperm count is still <5 million/ml after 6 months.
4. Perform seminal fluid analysis 1-3 monthly for another 6 months.
5. If the sperm count is >5-10 million/ml after 6 months, wait for spontaneous pregnancy.
6. If spontaneous pregnancy is not achieved, refer for ART.

Special thanks to Dr Rahilah binti Ahmad Shukri,
Reproductive Medicine Specialist, Hosp Sultanah Bahiyah, Alor Setar.

8 TESTS FOR HYPOGLYCAEMIA DISORDERS

8.1 72-hour fast/prolonged supervised fast

INDICATION

- To confirm and determine the cause of suspected spontaneous hypoglycaemia.

Contraindications

- Pregnancy
- Liver or kidney failure
- Unstable angina

Precautions

- Ensure that all patients are warned that they will be fasting, and are unable to leave the clinic/ward unaccompanied during the test.
- Ensure availability of Dextrose 50% and glucagon ampoule, in case of an emergency.

Preparation

- There is no special preparation required.
- The patient can have breakfast on the day of testing, if there is known frequent hypoglycaemic episodes.
- Prepare the materials:
  - 5 ml syringe to obtain blood samples
  - 50 ml syringes for Dextrose 50%
  - Branula
  - Needles
  - Ketone strips
  - Glucometer and strips
  - Intravenous (IV) Glucagon 1 mg

Specimen bottles

Investigation	Specimen bottle type
Serum Insulin	Plain tube *Yellow top with gel vacuum tube*
Serum C-peptide	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Sodium fluoride tube *Grey top with gel vacuum tube*

Method

1. Note the date of last ingestion of calories.
2. Document all baseline vital signs.
3. Stop all foods and drinks except for calorie-free and caffeine-free beverages and water.
4. Ensure that the patient is active during waking hours.
5. Insert the branula and flush it with 3-10 ml normal saline.
6. Perform a capillary blood glucose (CBG) using the following schedule:

CBG (mmol/L)	Frequency
≤4.0	Every hour
≤3.5	Every 30 minutes
≤3.0	Every 15 minutes

7. Once the CBG is ≤2.5 mmol/L or patient is symptomatic at ≤3.0 mmol/L, obtain blood samples for laboratory testing:
   a. Plasma glucose (3 ml)
   b. Insulin (3 ml)
   c. C-peptide (3 ml)
   d. Blood ketone
8. Check for capillary blood ketone every 8 hours; increasing ketone levels of >2.7 mmol/L indicate non-insulin mediated hypoglycaemia.
9. End the fast when any of the following occurs:
   a. The patient has symptoms or signs of hypoglycaemia when plasma glucose is ≤2.5 mmol/L.
   b. The plasma glucose (not CBG) concentration is ≤2.5 mmol/L in an asymptomatic patient after careful evaluation (see Caveats).
   c. The plasma glucose concentration is <3.0 mmol/L if Whipple's triad was documented on a prior occasion.
   d. 72-hours have elapsed.
10. Before ending the test, obtain blood samples for the following:
    a. Plasma glucose (3 ml)
    b. Insulin (3 ml)
    c. C-peptide (3 ml)
    d. Blood ketone
11. Administer IV 1 mg glucagon and recheck plasma glucose and blood ketone 10, 20 and 30 minutes after the IV glucagon.
12. Patient should then be fed right away after last blood draw.

Important notes

- The decision to end the fast must not be made on the basis of a CBG value.
- If it is judged necessary to treat urgently because of severe symptoms, obtain samples for 4 tests noted above before administering carbohydrates.
- It is recommended to obtain blood samples twice a day regardless of CBG levels. In some cases, the observed trends can help clinicians with interpreting the results.
- Samples can be kept at room temperature but must be sent to lab within 2 hours post-collection.
- Insulin and C-peptide samples will be analysed at a hospital with facilities for testing (see Appendix B for the list of hospitals)
- Analysis should only be done for the samples in which the plasma glucose is <2.5 mmol/L.

Caveats

- It is important that the blood samples and laboratory slips be carefully labelled, particularly with the exact time, and that the labelling information be recorded on a flow sheet. Interpretation of the results is possible only with these details.
- Young, lean, healthy women may have plasma glucose concentrations in the range of 2.2 mmol/L (40 mg/dl) or lower after prolonged periods of fasting.
- A low plasma glucose value is necessary but not sufficient for the diagnosis of hypoglycaemia.
  - **Do not stop the tests other than for the conditions specified in the procedure.**
  - Careful questioning and testing for subtle symptoms or signs of hypoglycaemia should be performed repeatedly when the patient's plasma glucose is near or in the hypoglycaemic range.
- The sensitivity and specificity of the established diagnostic parameters below are:
  - Insulin (≥3 µU/ml): 93% and 95 %
  - C-peptide (≥0.2 nmol/ml): 100% and 60 %
  - Plasma glucose response to IV glucagon (≥1.4 mmol/L): 91% and 95 %
- The **specificity** of these parameters improved when compared to normal patients whose plasma glucose at the end of a prolonged fast was 2.7 nmol/L or less.
  - Insulin: 100 %
  - C-peptide: 78 %
  - Plasma glucose response to glucagon: 100 %
- About 6 % of insulinomas and other disorders such as nesidioblastosis, can present as post-prandial hypoglycaemia. Mixed-meal tests may have a role in evaluating these patients.

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

**Circulating levels of sulphonylurea will confirm the presence of an oral glucose-lowering drug. **Presence of antibodies against insulin will confirm insulin autoimmunity.*
IGF, insulin-like growth factor; NIPHS, non-insulinoma pancreatogenous hypoglycaemia syndrome; OGLD, oral glucose-lowering drug; PGBH, post-gastric bypass hypoglycaemia.
Adapted from Cryer PE, et al. J Clin Endocrinol Metab 2009.

8.2 Mixed-meal test

INDICATION

- Patients with suspected post-prandial hypoglycaemia.

Preparation

- Patient should be fasted overnight for 10 hours before the test.
- Withhold all non-essential medications on the day of the test.
- On the day of the test:
  - Prepare Ensure® Plus high protein drink at 6 ml/kg with a maximum dose of 360 ml.*
  - Sufficient blood specimen bottles for testing with a maximum of 3 tubes for 1 testing set (see Specimen bottles below).
  - A syringe containing 50% glucose solution.
  - IV glucagon 1 mg.
  - Branula, syringes and needles.
  - Glucometer and strips.
  - Dextrose 50% and some fruit juice, in case of emergency.

**Some experts suggest using the same meals that trigger or provoke hypoglycaemic symptoms in the patient.*

Specimen bottles

Investigation	Specimen bottle type
Serum Insulin	Plain tube *Yellow top with gel vacuum tube*
Serum C-peptide	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Sodium fluoride tube *Grey top with gel vacuum tube*

Method

1. Have the patient consume the Ensure® Plus high protein drink.
2. Obtain a CBG and plasma glucose at 0 (baseline), 15, 30, 60, 90 and 120 minutes, and at the 3rd, 4th, and 5th hour.
3. Observe the patient for signs and symptoms of hypoglycaemia. In addition, ask the patient to keep a written log of all symptoms that are timed from the start of ingesting the Ensure® Plus high protein drink. If possible, avoid providing any carbohydrate or food until the test is completed.
4. Once the CBG is <3.3 mmol/L, obtain venous blood samples for plasma glucose, serum insulin and C-peptide. However, analyses for insulin and C-peptide are done only for samples with **plasma glucose** <3.3 mmol/L.
5. If the patient has severe hypoglycaemic symptoms and it becomes necessary to treat them, obtain samples for plasma glucose, insulin and C-peptide before administering carbohydrates.
6. Additionally, obtain samples to check for sulphonylurea (if assay for detecting sulphonylurea is available).

Minutes	0	15	30	60	90	120	180	240	300
CBG									
Plasma glucose (mmol/L)									
Insulin (µU/ml)									
C-peptide (pmol/L)									

Interpretation

Refer to the table for interpreting the 72-hour fast test (page 44). It is important to note that as the standards for interpreting the mixed-meal test have not been established, the current clinical utilisation of the test is to apply the same criteria for fasting conditions in insulinoma.

A symptomatic patient with a normal plasma glucose can be diagnosed with post-prandial syndrome.

Caveats

- An oral glucose tolerance test (OGTT) is not recommended for the evaluation of suspected post-prandial hypoglycaemia because the nadir for plasma glucose concentration after the ingestion of glucose may fall into the hypoglycaemia range in normal, asymptomatic individuals.
- However, some experts have recommended prolonged/modified OGTT as the preferred diagnostic test for dumping syndrome.
- Post-prandial (reactive) hypoglycaemia is a description of the timing of hypoglycaemia and is not a diagnosis.
  - When biochemical evidence of post-prandial hypoglycaemia is confirmed, the cause must be determined.
  - Using plasma glucose concentrations <3.3 mmol/L has been suggested as a sensitive cut-off value for meal-induced hypoglycaemia during OGTT or a mixed-meal test.

8.3 Arterial stimulation venous sampling (ASVS)/Selective arterial injection/Calcium stimulation test

INDICATION

- Localisation of an insulinoma.

Contraindications

- Allergy to contrast dye.
- Ischaemic heart disease.
- Orthopnoea.
- Severe bleeding tendency; if the patient is on aspirin or clopidogrel, discussion with the radiologist is required.

Precautions

- Stop diazoxide 7 days before the procedure as its elimination half-life is from 21-36 hours.
- Flushing and nausea may follow the calcium injection.
- Monitor the CBG and maintain levels at 3-5 mmol/L with dextrose infusion, if necessary.
- The risks of angiography include bleeding from sheath sites, thrombosis/dissection of femoral artery and visceral arteries, and dye allergy.

Preparation

- An interventional radiologist should take the consent from the patient.
- Blood for full blood count, urea and electrolytes, clotting parameters, and group & save for crossmatch should be done before the test.
- Prepare and carefully label a series of specimen bottles for each run; there should be enough for 4 runs and 2 spare runs).
- A glucometer and strips should be available.
- The patient should be fasted for at least 4 hours.
- Have a Dextrose 5% drip available to administer to the patient, in case of hypoglycaemia and to maintain CBG at 3-5 mmol/L.
- Calculate the dose of calcium for injection:

Each calcium injection should be **0.003125 mmol/kg body weight in 5 ml**	
Number of mmol calcium per aliquot	0.003125 x body weight in kg
Number of mmol calcium in 100 ml saline	0.003125 x body weight in kg x (100/5)
10% calcium gluconate contains 225 mmol calcium per 1000 ml so,	
Volume of 10% calcium gluconate needed is	(0.003125 x body weight in kg) x (100/5) x (1000/225) **= Body weight in kg x 0.2778** **= x ml of calcium gluconate**

- Remove the calculated volume from a 100 ml saline bag and replace with equivalent volume of calcium gluconate.
- Draw up and inject in 5 ml aliquots.

Specimen bottles

Investigation	Specimen bottle type
Serum Insulin	Plain tube *Yellow top with gel vacuum tube*
Serum C-peptide	Plain tube *Yellow top with gel vacuum tube*
Plasma glucose	Sodium fluoride tube *Grey top with gel vacuum tube*

Method

1. Routinely, 2 separate catheters are inserted into the femoral vein and artery at the groin level.
2. Under fluoroscopic guidance, the venous catheter is positioned in the right hepatic vein for blood sampling.
3. Highly selective angiography is then performed with selective catheterisation of the right hepatic vein.
4. Following angiography, each artery (proximal and distal splenic arteries, superior mesenteric artery, right hepatic artery, gastroduodenal artery, and left hepatic artery) is re-catheterised in turn, preferably starting with the vessels least likely of supplying the tumour. Occasionally, the dorsal pancreatic artery is also catheterised.
5. Obtain 2 baseline samples for plasma glucose at time (T) -120 and 0 seconds. Note the results.
6. At T0, rapidly inject a calcium gluconate bolus into the artery.
7. Obtain blood samples as T30, 60, 90, 120 and 180 seconds; call out a 10 second countdown before each sample.
8. Check the plasma glucose of the T180 second sample.
9. Samples for insulin and C-peptide should be separated within 30 minutes, and stored on ice if the procedure is prolonged.

Time (seconds)		0	30	60	90	120	180
ARTERY							
Prox Sp A	CBG						
	INS/CPEP						
Dis Sp A	CBG						
	INS/CPEP						
SMA	CBG						
	INS/CPEP						
RHA	CBG						
	INS/CPEP						
GDA	CBG						
	INS/CPEP						
LHA	CBG						
	INS/CPEP						

Prox Sp A, proximal splenic artery; Dis Sp A, distal splenic artery; SMA, superior mesenteric artery; RHA, right hepatic artery; GDA, gastroduodenal artery; LHA, left hepatic artery; INS/CPEP, insulin/C-peptide; CBG, capillary blood glucose.

Interpretation

- The criterion for localisation is a 2-fold rise in insulin in the 30- or 60-second hepatic vein samples following injection into the relevant arterial territory.

9 TESTS FOR PRIMARY ALDOSTERONISM

The investigation for primary aldosteronism will require an initial biochemical screening of individuals at risk, followed by confirmatory biochemical testing for those with a positive screening test. Subsequently, confirmed cases will require localisation tests, which include a combination of imaging studies and hormonal venous sampling.

9.1 Screening test: Aldosterone renin ratio (ARR)

The definition of ARR is:

$$\frac{\text{Plasma aldosterone concentration (PAC) (pmol/L)}}{\text{Plasma renin activity (PRA) (ng/ml/hour) or Direct renin concentration (DRC) (mU/L)}}$$

INDICATIONS

- Sustained blood pressure (BP) >150/100 mmHg on 3 different days.
- BP >140/90 mmHg despite being on 3 different anti-hypertensive drugs, including a diuretic.
- Controlled BP (<140/90 mmHg) on 4 or more anti-hypertensive drugs.
- Hypertension with spontaneous or diuretic-induced hypokalaemia, adrenal incidentaloma or sleep apnoea.
- Hypertension with a family history of early onset hypertension or cerebrovascular accident (CVA) at <40 years old.
- All first-degree relatives of patients with primary aldosteronism with hypertension.

Preparation

- Withdraw anti-hypertensive drugs (e.g. aldosterone antagonists and diuretics) and liquorice derivatives that can significantly affect the ARR ≥4 weeks before the test.
- In female patients, establish the patient's oral contraceptive pills or estrogen hormone replacement status as estrogen-containing medications may lower DRC.
- Correct any hypokalaemia and maintain a target serum potassium of 4-4.5 mmol/L.
- Allow patient to have liberalised salt intake while maintaining a normal hydration status.

9. Tests for Primary Aldosteronism

Specimen bottles

Investigation	Specimen bottle type
Plasma renin	EDTA tube *Purple top with gel vacuum tube*
Plasma aldosterone	EDTA tube *Purple top with gel vacuum tube*
Serum potassium	Plain tube *Yellow top with gel vacuum tube*

Method

1. Obtain blood for renin and aldosterone mid-morning (8 a.m.–10 a.m.) after the patient has been up (sitting, standing, or walking) for at least 2 hours and seated for 5–15 minutes after.
2. If the initial ARR is of borderline significance and BP can be controlled without tolerability issues using medications with minimal effect on ARR (Table), consider withdrawing other agents that moderately affect ARR (e.g., beta blockers, central α-2 agonists, angiotensin-converting enzyme [ACE] inhibitors/angiotensin receptor blockers [ARBs], renin inhibitors, dihydropyridine calcium channel blockers [CCBs], and non-steroidal anti-inflammatory drugs [NSAIDs]) for ≥2 weeks.
3. Complete the laboratory form that includes a comprehensive history, indication for testing, concurrent anti-hypertensive drugs, and potassium values, which is signed by a specialist/endocrinologist.
4. All samples should be sent to the lab immediately for processing, i.e., within 30 minutes from collection.

Medications that have minimal interference on the ARR

Medications	Dose
Non-dihydropyridine CCBs	
• **Verapamil**	40-240 mg bd/tds
• **Verapamil SR**	90-120 mg od/bd
α-blockers	
• **Prazosin**	0.5-5 mg bd/tds/qid
• **Terazosin**	1-20 mg od
• **Doxazosin**	1-16 mg od
Vasodilators	
• **Hydralazine**	10-50 mg bd/tds/qid
α-2 adrenergic agonist	
• **Moxonidine (Physioten)**	0.2 - 0.4 mg od

*Interpretation**

$$\text{ARR} = \frac{\text{PAC (pmol/L)}}{\text{DRC (mU/L)}}$$

- < 25 – negative (PA is highly unlikely)
- 25 - 35 - indeterminate
- > 35 - positive (PA highly likely)
- Primary aldosteronism can be excluded if PAC is < 170 pmol/L (6 ng/dl) regardless of renin value.

** According to IDS - iSYS® automated assay system.*
All cut off values may differ according to the assays used. Please refer to the local laboratory reports
Aldosterone unit conversion : 1 ng/dL = 27.74 pmol/L

9.2 Confirmatory tests

9.2.1 Saline suppression test (SST)

INDICATION

- Confirmation of primary aldosteronism.

Contraindications

- History of congestive cardiac failure (CCF) or fluid overload.
- Advanced chronic kidney disease (CKD).
- Severe uncontrolled hypertension (>180/110 mmHg).
- Severe hypokalaemia.
- Cardiac arrhythmias.

Specimen bottles

Investigation	Specimen bottle type
Plasma aldosterone	EDTA tube *Purple top with gel vacuum tube*
Plasma renin	EDTA tube *Purple top with gel vacuum tube*

Method

1. Utilise anti-hypertensive drugs with minimal or no effect on renin-angiotensin aldosterone secretion (refer to the table Medications that have minimal interference on the ARR on page 50).
2. Check the plasma potassium stat upon the patient's arrival.
3. Avoid administration of potassium chloride in the form of intravenous (IV) infusion in normal saline 0.9% after 12 midnight.
4. Start the test at 8 a.m. the next morning.
5. Infuse IV normal saline 0.9% at 500 ml/hour x 4 hours using an infusion pump (total infusion is 2 L over 4 hours) to patient in recumbent position.
6. Monitor the BP, pulse rate and for signs of fluid overload during the procedure.
7. Send a sample for plasma aldosterone level after completing the normal saline infusion.
8. Complete the laboratory form that includes a comprehensive history, indication for testing, concurrent anti-hypertensive drugs, and potassium values, which is signed by a specialist/ endocrinologist. Indicate the sample as post-saline suppression.

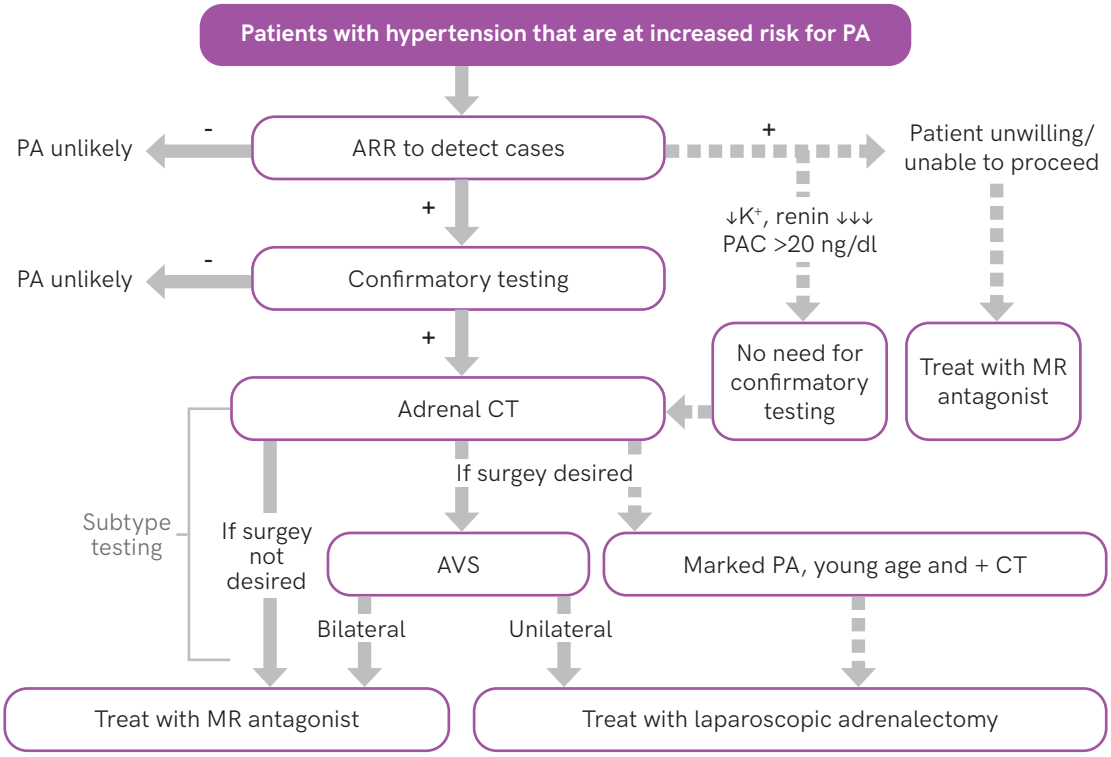
Note:
- *This procedure can be performed on an outpatient basis in a day care setting for low-risk patients with optimised plasma potassium levels.*
- *The confirmatory test can be skipped in cases of spontaneous hypokalaemia with PAC > 550 pmol/L (20 ng/dl) and undetectable renin levels.*

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.

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.

9.2.2 Fludrocortisone suppression test (FST)

Fludrocortisone is a synthetic mineralocorticoid, which binds to mineralocorticoid receptors leading enhanced sodium uptake in epithelial tissues. Water follows the sodium causing increased volume load and cardiac output, ultimately leading to the development of increased BP and hypokalaemia.

INDICATION

- Confirmation of primary aldosteronism.

Contraindications

- CCF
- Uncontrolled hypertension
- Cardiac arrythmias

Precaution

- Watch for worsening hypertension, fluid overload and hypokalaemia during the test.
- Patients with renal impairment can give false negative results.

Specimen bottles

Investigation	Specimen bottle type
Plasma aldosterone	EDTA tube *Purple top with gel vacuum tube*
Plasma renin	EDTA tube *Purple top with gel vacuum tube*
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*
Serum potassium (blood urea and serum electrolytes [BUSE]	Plain tube *Yellow top with gel vacuum tube*

Method

1. The patient is admitted to the medical ward.
2. Ensure that the patient receives a high salt diet. An addition of slow-release sodium chloride 30 mmol/L 3-times a day has been suggested to achieve a urinary sodium excretion of 3 mmol/L/kg/day.
3. Administer oral fludrocortisone 0.1 mg every 6 hours for 4 days.
4. Add Slow K supplementation every 6 hours and monitor BUSE to keep the plasma potassium close to 4 mmol/L.
5. Continue to measure the serum potassium daily or 2-times daily throughout the test to ensure normokalaemia.
6. On Day 5, obtain blood samples for serum cortisol at 7 a.m. and 10 a.m., followed by a sample for plasma aldosterone at 10.00 a.m. Label the specimen tube correctly (Plasma Aldosterone-post-fludrocortisone suppression).
7. In addition, obtain a sample for serum BUSE to ensure the potassium level is normal.
8. Complete the laboratory form that includes a comprehensive history, indication for testing, concurrent anti-hypertensive drugs, and potassium values, which is signed by a specialist/endocrinologist. Indicate the sample as post-fludrocortisone suppression.

Day	Time	Samples for			
		Aldosterone	Renin	Cortisol	2-times daily BUSE for potassium
D1	Oral tablet fludrocortisone 0.1 mg every 6 hours started at 12 p.m., and continued up to D4.				
	-	-	-	-	✔
D2	-	-	-	-	✔
D3	-	-	-	-	✔
D4	-	-	-	-	✔
D5	7 a.m. (recumbent)	✔	✔	✔	✔
	10 a.m. (upright)	✔	✔	✔	✔

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.

9.2.3 Adrenal venous sampling (AVS)

AVS is an invasive and expensive test, making appropriate patient selection essential. It is a highly specialised, technically challenging procedure that requires multidisciplinary involvement. The aim of AVS is to subtype primary aldosteronism (PA) into either bilateral or unilateral idiopathic hyperaldosteronism (IHA), often caused by an aldosterone-producing adenoma (APA).

INDICATIONS

- In confirmed cases of PA with **normal adrenal glands, bilateral adrenal gland enlargement, adrenal micronodularity, or a unilateral adrenal nodule with discordant imaging and postural test results**, proceed to AVS only after a team discussion and a combined decision to proceed with this test.
- Confirmed PA patients who wish to pursue surgical management if results indicate unilateral hyperaldosteronism.

Preparation

- The appointment for an AVS is made by contacting the Interventional Radiologist in the respective hospital, and should be arranged as the 1st case in the morning, i.e., by 9 a.m.
- 6 weeks before the test: Stop spironolactone, amiloride and diuretics. Hypertension may be controlled with α-blockers (prazosin or terazosin) or CCBs (verapamil is preferred) or oral hydralazine.
- Within 1 week of the test: Check serum potassium to ensure normokalaemia. The serum potassium should preferably be >4.0 mmol/L before the test. Patients may require oral potassium supplementation with Slow K or potassium chloride mixture.
- One day before the test:
  - Admit the patient and ensure normokalaemia, optimised BP and obtain blood samples for full blood count, prothrombin time and partial thrombin time.
  - Insert a branula and keep the patient fasted overnight.
  - Obtain the consent for the AVS procedure.
  - Prepare and correctly label the specimen bottles.
  - Order for Synacthen® (Adrenocorticotropic hormone [ACTH] – Cosyntropin® 250 µg) from the pharmacy.
  - Inform the endocrine laboratory once the patient is admitted and arrange for laboratory staff to be present on site after sampling to transport the blood samples.
  - Make the most recent contrast-enhanced computed tomography (CT) adrenal available for the Interventional Radiologist to review.

Specimen bottles

Investigation	Specimen bottle type
Plasma aldosterone	EDTA tube *Purple top with gel vacuum tube*
Serum cortisol	Plain tube *Yellow top with gel vacuum tube*

Method

1. One hour before the test: Patient should lie down in supine position.
2. 30 minutes before the test: Prepare the ACTH IV infusion by diluting 250 µg Synacthen®/ Cosyntropin® into 500 ml Dextrose 5%.*
3. Prepare the infusion pump – Start the infusion 30 minutes before the procedure at a rate of 50 µg /hour or 100 ml/hour and continue the infusion throughout procedure.**
4. Cannulation is done via the transfemoral access.
   a. **Sequential** AVS is the method usually used where a SINGLE femoral vein access will be made by the Interventional Radiologist.
   b. Dual femoral vein access is called **Simultaneous** AVS.
5. **Sequential** AVS involves sampling from 1 adrenal vein, then the opposite adrenal vein, followed by peripheral sampling. For example: right adrenal vein, then left adrenal vein, and finally peripheral blood or infrarenal inferior vena cava (IVC). Samples are taken for PAC and serum cortisol, into the pre-labelled bottles.
6. **Simultaneous** AVS involves sampling of the right adrenal vein and left adrenal vein, IVC, or peripheral sample from the antecubital vein (PV) for PAC and serum cortisol by individually designated staff for each sampling site, using pre-labelled bottles.

** Synacthen®/Cosyntropin® is given during AVS to minimise stress-induced fluctuations in aldosterone secretion during sequential AVS, maximise the cortisol gradient between the adrenal vein and IVC (mixed venous) samples, and maximise aldosterone secretion in a unilateral adenoma, as most adenomas are partially ACTH-sensitive.*
***Note that Synacthen®/Cosyntropin® can also be given as a 250 µg bolus dose during the AVS.*

Choice between simultaneous or sequential catheterisation

The pulsatile secretion of aldosterone can generate time-related variability in hormone concentrations. Consensus guidelines suggest:

- If Cosyntropin® (ACTH) stimulation is used, the sequential technique is acceptable but higher selectivity index (SI) and lateralisation index (LI) thresholds are indicated.
- If Cosyntropin® (ACTH) stimulation is not used, then bilateral simultaneous AVS should be performed.

Safety and complications of AVS

In experienced hands, AVS is safe, with a very low complication rate, ≤2.5%. Complications of AVS include:

- Adrenal vein rupture: The rate was 0.61% in the AVIS Phase I trial
- Adrenal vein dissection
- Adrenal infarction
- Intraglandular/periadrenal/groin hematoma
- Femoral/adrenal vein thrombosis

Interpretation

Selectivity index (SI) is the ratio of the adrenal vein cortisol to the peripheral vein cortisol, and is used to ensure the correct placement of the catheters in both the adrenal veins. It is obtained by taking samples for cortisol from both the adrenal veins and compared with the cortisol level from the peripheral sample.

- SI ≥5 indicates successful catheterisation of the adrenal vein(s).
- SI ≥2 indicates successful catheterisation of the adrenal vein(s) when Cosyntropin® (ACTH) has not been used.
- SI <2 indicates that there was failure to catheterise 1 of the adrenal veins and all data from the analyses should be discarded.

Lateralisation index (LI) is the ratio of the ipsilateral aldosterone/cortisol (A/C) to the contralateral A/C ratios.

- LI ≥4.0 (ACTH-stimulated) or ≥2.0 (non-ACTH stimulated) denotes unilateral APA.

Contralateral suppression (CS) is the ratio of the PAC and plasma cortisol concentration (PCC) of the non-dominant side to the PAC and PCC of the IVC ratios ($PAC_{non\text{-}dom}/PCC_{non\text{-}dom}$: PAC_{IVC}/PCC_{IVC})

- An AC ratio lower on the non-dominant side compared to the AC ratio of IVC/peripheral values suggests suppression of aldosterone production in the non-dominant adrenal and overproduction in the dominant adrenal.
- If LI is ≥ 4.0, CS is NOT required to refer patients for adrenalectomy.
- In situations where data is incomplete (1 adrenal vein not catheterised, SI <2), if the PAC/PCC ratio in the adequately sampled adrenal vein specimen is convincingly lower than that of the IVC (CS <1), it can be presumed that the majority of aldosterone is derived from the unsampled adrenal vein by exclusion. Here, the removal of the latter adrenal is justified.

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

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/cortisol ratio; AV, adrenal vein; AV/P, adrenal vein/peripheral vein ratio; SI, selectivity index.

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).

Precaution

- Patient should be continuously supervised during the procedure.

Preparation

- Patient should be fasted overnight for 10 hours.
- Hold all non-essential medications.
- Measure the patient's weight and height to calculate the body mass index (BMI).

Specimen bottle

Investigation	Specimen bottle type
Thyroid function test or free thyroxine (FT4)	Plain tube *Yellow top with gel vacuum tube*

Method

1. Insert a branula and flush with 3-10 ml normal saline, as necessary.
2. Obtain baseline blood sample for total T4 or FT4 levels.
3. Have the patient consume 1000 µg of LT4, and document the dose and time.
4. Obtain blood samples for total T4 or FT4 levels hourly for 5 hours. Document the laboratory results with the sample collection times.
5. Monitor the patient's blood pressure (BP) and heart rate hourly.

Example of documenting the test

Dose:		Time:		Date:		
Weight:		Height:		BMI (kg/m^2):		
Time (minutes)	**Baseline**	**60**	**120**	**180**	**240**	**300**
FT4 (pmol/L)						

Interpretation

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).

- *A urine osmolality of >800 mOsm/kg indicates optimal plasma arginine vasopressin (AVP) levels and appropriate renal response to AVP, therefore ruling out any form of DI.*
- *If the baseline plasma osmolality >295 mOsm/kg, plasma sodium >145 mmol/L and urine osmolality is <300 mOsm/kg, PP is excluded. Proceed with a desmopressin (DDAVP) challenge test.*

Preparation

- Do not perform the test in untreated anterior pituitary hormone deficiency, as steroid and thyroxine deficiencies impair excretion of a free water load.
- Exclude other causes of polyuria before the test: diuretics, hypercalcaemia, hypokalaemia, hyperglycaemia, and renal disease.
- Stop interfering medications at least 24 hours earlier (e.g. diuretics, and DDAVP). Give normal hormone replacements before test.
- Patient should omit tobacco and alcohol consumption the night before the test.
- There is no need to fast and the patient can be allowed a light breakfast.

Specimen bottle

Investigation	Specimen bottle type
Renal profile/BUSE	Plain tube *Yellow top with gel vacuum tube*
Serum osmolality	Plain tube *Yellow top with gel vacuum tube*
Urine osmolality	Urine specimen container

Method

1. The patient should not be allowed any fluids, but dry food (e.g. toast) is permitted.
2. The patient's weight and blood pressure (BP) at baseline, 0 minute (T0), should be recorded. If available, obtain plasma AVP/Copeptin levels.
3. T0 urine should be passed and discarded and then collected hourly for volume and osmolality measurements.
4. Repeat BP measurements hourly; repeat weight measurement and obtain blood for plasma sodium and osmolality every 2 hours.

5. The test ends after 8 hours. It may be stopped earlier if:
   a. There is >3-5% weight loss.
   b. Plasma sodium is >upper limit of normal (ULN).
   c. Urine osmolality >800 mOsm/kg
   d. Development of hypotension (systolic BP ≤100 mmHg)
6. If available, obtain sample for plasma AVP/Copeptin at the end of the test.
7. The patient can be allowed to eat and drink freely after the test.
8. Administer DDAVP 2 µg subcutaneously or 20 µg intranasally, and continue measuring the urine volume and osmolality every hour for another 3 hours.

Example of documenting the test

Name: Patient ID:
NRIC: Ward:
Date:

Weight:
3% body weight (kg): 5% body weight (kg):
Weight must not fall below _____ kg

Time point (minutes)	Time	BP (mmHg)	Urine volume (ml)	Plasma osmolality (mOsm/kg)	Urine osmolality (mOsm/kg)	Renal profile (blood urea and sodium)	Weight (kg)
0 (baseline)	0800-0900	*	*	*	*	*	*
60	0900-1000	*	*		*		
120	1000-1100	*	*	*	*	*	*
180	1100-1200	*	*		*		
240	1200-1300	*	*	*	*	*	*
300	1300-1400	*	*		*		
360	1400-1500	*	*	*	*	*	*

11. Water Deprivation Test (WDT)

Time point (minutes)	Time	BP (mmHg)	Urine volume (ml)	Plasma osmolality (mOsm/kg)	Urine osmolality (mOsm/kg)	Renal profile (blood urea and sodium)	Weight (kg)
420	1500-1600	*	*		*		
480	1600-1700	*	*	*	*	*	*
IV or SC DDAVP 2µg – allow patient to eat and drink freely							
	1700-1800		*		*		
	1800-1900		*		*		
	1900-2000		*		*		

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.

INDICATION

- To demonstrate or confirm autonomic neuropathy.

Contraindications

- Proliferative retinopathy – The Valsalva manoeuvre should not be performed in patients with proliferative retinopathy because of the risk of retinal haemorrhage.
- Atrial fibrillation – The tests will be uninterpretable, except for the postural hypotension and hand grip tests.

Preparation

- Sphygmomanometer for blood pressure (BP) measurement.
- Mouthpiece to attach to sphygmomanometer (5 ml syringe minus plunger) or aneroid manometer (refer to diagram).
- An old-fashioned electrocardiogram (ECG) machine that can record long strips.

Method

Tests for cardiac parasympathetic damage

1. Heart rate response to the Valsalva manoeuvre:
   a. Start the ECG machine (limb leads only, use lead II).
   b. Patient blows into the sphygmomanometer and maintains the pressure at 40 mmHg for 15 seconds, continue recording for 30 seconds after release of pressure. Mark these points on the ECG.
   c. Measure the shortest R-R interval during manoeuvre and the longest after.
   d. Valsalva ratio = longest after/shortest during.
   e. Take the mean of 3 readings.
2. Heart rate variation during deep breathing:
   a. Start the ECG machine.
   b. Ask the patient to breathe quietly at a rate of 6 breaths over 1 minute (5 seconds in and 5 seconds out).

c. Mark the ECG at the start of each inspiration and expiration.
d. Measure the maximum and minimum R-R interval for each cycle and convert to beats/minute.
e. Result is the mean difference (max - min) for heart rate during deep breathing.

3. Heart rate response to standing:
   a. Start the ECG recording with the patient lying.
   b. Ask the patient to stand and, mark this moment on the ECG. Continue recording the ECG for 1 minute.
   c. Measure the shortest R-R interval around the 15th beat after standing and the longest around the 30th beat. Calculate the 30:15 ratio by dividing the longest by the shortest interval.

Tests for sympathetic dysfunction

1. BP response to standing:
   a. Measure the BP with the patient lying down and subsequently, 2 minutes after standing. Record the postural difference.
2. BP response to sustained hand grip:
   a. Use 2 manometers/sphygmomanometers.
   b. First, with the subject at rest, record the BP while lying supine.
   c. Then, ask the subject to grip the inflated cuff of a manometer at 30% of maximum voluntary contraction for 2 minutes. Just before the grip is released, BP is recorded on the opposite arm.
   d. The difference between the diastolic BP just before release and before the hand grip is a measure of the response.

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

12. Tests to Assess for Autonomic Neuropathy

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

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 2
Short Synacthen® Test (SST)

Short Synacthen® Test (SST)

Name: **Patient ID:**
Date:

Time (minutes)	Cortisol (nmol/L)
0	
Inject IV or IM Synacthen® 250 µg	
30	
60	

IM, intramuscular; IV, intravenous.

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 4
Glucagon Stimulation Test (GST)

Glucose Stimulation Test (GST)

Date:
Name: **Patient ID:**
Age: **Sex:**
Weight (kg): **Height (m):** **BMI (kg/m²):**

Glucagon dose:

Diabetes mellitus: ☐ Yes ☐ No

Time (minutes)	CBG (mmol/L)	GH (µg/L)	Venous BG (mmol/L)
0			
Inject IM glucagon			
60			
90			
120			
150			
180			
210			
240			

BG, blood glucose; CBG, capillary blood glucose; GH, growth hormone; IM, intramuscular.

Appendix 5
Thyrotrophin Releasing Hormone (TRH) stimulation test

Thyrotrophin Releasing Hormone (TRH) Stimulation Test

Name: **Patient ID:**
Date:

Test/Time (minutes)	0	30	60
TSH (mIU/L)			
FT4 (pmol/L)		-	-

FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroxine stimulating hormone.

Appendix 6
Gonadotrophin-relating Hormone (GnRH) or Luteinising-hormone-releasing-hormone (LHRH) stimulation test

Gonadotrophin-relating Hormone (GnRH) or Luteinising-hormone-releasing-hormone (LHRH) stimulation test

Name: **Patient ID:**
Date:

Time (minutes)	FSH (U/L)	LH (U/L)
0		
Inject IV GnRH or LHRH		
30		
60		

FSH, follicular stimulating hormone; IV, intravenous; LH, luteinising 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 8
Mixed-Meal Test

Mixed-Meal Test

Name: **Patient ID:**
Date:

Time start:

Type of mixed meal:

Test/Time (minutes)	0	15	30	60	90	120	180	240	300
CBG (mmol/L)									
Plasma glucose (mmol/L)									
Insulin (µU/ml)									
C-peptide (pmol/L)									

CBG, capillary blood glucose.

Appendix 9
Arterial Stimulation Venous Sampling (ASVS)

Arterial Stimulation Venous Sampling (ASVS)

Name: **Patient ID:**
Date:

Calcium gluconate dose:

Time start:

Site/Time (seconds)	Test	0	30	60	90	120	180
Proximal splenic artery	CBG (mmol/L)						
	INS/CPEP						
Distal splenic artery	CBG (mmol/L)						
	INS/CPEP						
Superior mesenteric artery	CBG (mmol/L)						
	INS/CPEP						
Right hepatic artery	CBG (mmol/L)						
	INS/CPEP						
Gastroduodenal artery	CBG (mmol/L)						
	INS/CPEP						
Left hepatic artery	CBG (mmol/L)						
	INS/CPEP						

CBG, capillary blood glucose; INS/CPEP; insulin to C-peptide ratio.

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 11
Levothyroxine Absorption test

Levothyroxine Absorption Test

Name: **Patient ID:**
Date:
Weight (kg): **Height (m):** **BMI (kg/m²):**

Levothyroxine (LT4) dose administration
Dose: **Time:** **Date:**

Test/Time (minutes)	Baseline	60	120	180	240	300
FT4 (mmol/L)						

FT4, free thyroxine.

Appendix 12
Water Deprivation Test (WDT)

Water Deprivation Test

Name: **Patient ID:**
Date:
Weight:
3%-5% weight (kg):
Weight must not fall below: **kg**

Time point (minutes)	Time	BP (mmHg)	Urine volume (ml)	Plasma osmolality (mOsm/kg)	Urine osmolality (mOsm/kg)	Renal profile (blood urea and sodium)	Weight (kg)
0 (baseline)	0800-0900						
60	0900-1000			-		-	-
120	1000-1100						
180	1100-1200			-		-	-
240	1200-1300						
300	1300-1400			-		-	-
360	1400-1500						
420	1500-1600			-		-	-
480	1600-1700						
IV or SC DDAVP 2µg – allow patient to eat and drink freely							
	1700-1800			-		-	-
	1800-1900			-		-	-
	1900-2000			-		-	-

BP, blood pressure; DDAVP, Desmopressin; IV, intravenous; SC, subcutaneous.

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

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