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Baseline pituitary functions:

Investigations done **8-10 AM**

1. FT4, TSH
2. Prolactin
3. LH/FSH, Testosterone/Estradiol,SHBG
4. IGF1
5. Plasma& Urine osmolality
6. Serum Cortisol

Note:

1. In cases of suspected pituitary insufficiency, a basal morning serum cortisol of < 100 nmol/l strongly indicates ACTH deficiency and therefore dynamic testing is not necessary to confirm diagnosis and glucocorticoid replacement should be started immediately and dynamic tests organised later.
2. If the basal serum cortisol is between 100-450 nmol/l and/or GHD suspected , then proceed to ITT or prolonged glucagon test.
3. TSH alone may be misleading in pituitary disease
4. If any of the above tests are abnormal, proceed to pituitary imaging

TRH test:

Indications:

- 1.To diagnose preclinical primary hypothyroidism who show an exaggerated TSH response.
- 2.In those with central hypothyroidism allowing the differentiation between pituitary and hypothalamic hypothyroidism.
- 3.May be used in the diagnosis of subclinical primary hyperthyroidism.(generally unnecessary since the introduction of ultra sensitive TSH assays) but may be necessary where baseline TSH is variable.
- 4.Added test in hyperprolactinemia to discriminate functional hyperprolactinemia from microprolactinoma.(not done routinely especially with the availability of MRI).
- 5.To distinguish between TSH omas(usually unresponsive) and Thyroid resistance states.(always responsive)
6. Helpful alternative test in acromegaly with Growth Hormone measurements when other results are equivocal.

Patient preparation:

1. No fasting necessary. Light breakfast (tea / toast)
2. Weigh patient
3. Baseline BP and pulse.
4. Patients should be off thyroxine for 3 weeks prior to test , though this test is very rarely used on patients taking thyroxine

Dose:

200 micrograms TRH iv

Measure baseline T4 and TSH at time 0 and TSH at 20, 60 min +/- prolactin/GH

Precautions:(better not to do)

Coronary artery disease.

Side effects:

Mild and transient. nausea, desire to micturate, flushing, dizziness and peculiar taste.Lasts a few minutes, best done when lying supine.

Interpretation:

1. Normal response is rise in TSH by more than 2 mu/l to > 3.4mu/l but less than 22 mu/l with a maximum at 20 min and lower values at 60 min.

Typical responses in thyroid disease:

Time	TSH mu/l				
	Normal	Hypothy	Hyperthy	Hypopit	Hypothalam defect
0 min	0.4-4	5-50	0.1	1	1
20 min	5-22	22-90	0.1	3	4
60 min	3-15	15-80	0.1	2	7

These are rough guides only and gives a pattern of defects than absolute values. In Ninewells the cutoff is taken at 22 mu/l of TSH for primary preclinical hypothyroidism.

Oestrogens, theophylline and levodopa enhance the response and steroids depress response.

2. If the rise in prolactin is <100 % , then this is thought to indicate a tumour. In normal and hyperfunctional states prolactin rise is more than 100%(usually 3 –5 fold)

3. The normal GH response at 20 and 60 min following TRH is GH suppression, however 80% of patients with acromegaly show an increase (by 50% of basal).

Generally:

1. A delayed peak (60 min rather than 20) is typically found in hypothalamic disease.
2. In hyperthyroidism there is no TSH response to TRH.
3. TRH test is useful in differentiating RTH from TSHomas. Circulating TSH shows a normal or exaggerated response to TRH that is suppressed following T3 administration (Werner's test: 80-100 mcg orally of T3 for 8-10 days) in patients with RTH whereas TSH secretion from autonomous tumours is unresponsive.

Check list for raised prolactin levels:

1. Repeat prolactin levels
2. Drug history
3. Pregnancy test
4. Baseline pituitary function tests inc TSH
5. Renal functions
6. Dynamic tests if necessary
7. Think of PCOS, Macroprolactin and biochemical hook effect
8. MRI pituitary if indicated
9. Visual field testing for macroprolactinomas

Biochemical hook effect:

This occurs where the assay utilizes antibodies recognizing 2 ends of the molecule. One is used to capture the molecule and one to label it. If PRL levels are very high, it may be bound to one antibody and not to the other. Thus above a certain concentration (200,000mU/L), the signal will reduce rather than increase and very high PRL levels will be spuriously reported as normal. This effect is identified by requesting measurement of the sample at dilution.

Macroprolactin:

Occasionally, larger forms of prolactin are detected in circulation (macroprolactin) but does depend on the immunoassay used. Macroprolactin does not interfere with reproductive function. All specimens with measured prolactin above the normal range will be screened for macroprolactin with the monomeric prolactin reported.

LHRH Test:

This test is seldom useful and gonadotropin deficiency is diagnosed on the basal levels rather than dynamic response. This test has a low sensitivity and specificity.

Principle of the test:

GnRH is a hypothalamic decapeptide which stimulates the release of both FSH and LH. The main use is in the assessment of patients with suspected hypothalamic/pituitary failure often combined with TRH test.

Indications:

1. To further investigate possible gonadotropin deficiency.
2. To confirm precocious puberty.

Note:

1. The test is inappropriate in post menopausal females with elevated basal level of gonadotropins.
2. In females with normal menstrual cycles, this test should be performed in follicular phase (day 3-7 of the cycle)

Procedure:

1. Overnight fast not necessary if done alone. Light breakfast.
2. Site indwelling canula
3. Take baseline bloods: LH, FSH and testosterone (M) or oestradiol (F).
4. 100 mcg LHRH (GnRH - Gonadorelin) intravenously.
5. Flush cannula with saline.
6. Take samples for LH and FSH at t = 30 and 60 mins.

Interpretation:

LH levels usually increase 4-10 fold above basal. (usually exceeds 10u/l). The normal peaks occur at 30 or 60 mins. LH response normally greater than FSH.

Minimal increase is ≥ 2 U/L in FSH and ≥ 5 U/L in LH

An inadequate response may be an early indication of hypopituitarism.

Normal, subnormal and exaggerated responses may be seen in hypothalamic disease.

Prepubertal children should have no response of LH or FSH to LHRH. If sex steroids are present (patients undergoing precocious puberty) the pituitary will be primed and will therefore respond to LHRH.

Insulin tolerance test:

Gold standard for assessing the integrity of the hypothalamo-pituitary-adrenal axis.

Reproducibility among healthy volunteers is well documented but not known amongst patients with pituitary disease.

The assumption that the ability to respond to insulin induced hypoglycemia will translate into appropriate cortisol rise in the event of acute illness or major surgery is supported by studies in which the peak cortisol levels of patients undergoing major surgery were comparable to those achieved during a preoperative ITT.

Indications:

1. Assessment of ACTH and Cortisol reserve.
2. Assessment of GH reserve.
3. Differentiation of Cushing's syndrome from depression.

Contra Indications:

1. Epilepsy
2. Ischaemic heart disease or abnormal ECG
3. Untreated hypothyroidism

Note:

Patients should discontinue oestrogen replacement for 6 weeks before the test as increased CBG will make cortisol results difficult to interpret.

Calculating Actrapid dose:

Normal pituitary function: 0.15U/ kg

Acromegaly, Cushing's, 0.3u/kg

Hypopituitary 0.1u/kg

Diabetes mellitus 0.2 u/kg

Always ensure that 50 mls of 50% dextrose or 100 mls of 20 % dextrose and iv hydrocortisone available during the test. Must ensure high dose dextrose injections do not extravasate.

Timing of samples:

0 min, 30, 60, 90, 120 minutes for **Glucose, Cortisol, GH**
Protocol: See attached CIU sheet

Note:

If after 45 minutes the blood glucose level is not < 2.2 mmol/l and patient not clinically hypoglycemic, repeat the insulin injection at half dose and sample at 75 min and 150 min as well as other samples.

With severe and prolonged hypoglycemia or impending or actual LOC or fits, it may be necessary to terminate the hypo. Give 10-20 mls of 50% dextrose IV bolus followed by 5% dextrose infusion at 100 ml/hr. Importantly continue sampling if possible as the hypoglycemic stimulus has been adequate. Consider 4 mg dexamethasone at the end of the test if actual LOC or fit.

There must be atleast 2 specimens following adequate hypoglycemia. This does not mean that the patient needs to be hypoglycaemic for that length of time

Interpretation:

1. A normal response is for the cortisol to rise > 170 nmol/l to a peak of > 550 nmol/l.

2. Serum GH levels rises to > 20 mU/L

3. If adequate hypoglycemia was not achieved (< 2.2 mmol/L), cortisol or GH deficiency cannot be diagnosed. It may be necessary to repeat the test with a larger insulin dose.

4. A normal cortisol response demonstrates ability to withstand stress including major surgery without steroid cover.

5. A subnormal response (peak cortisol 450-570) may be managed by administration of steroids at the time of stress only.

6. Other patients with subnormal responses need glucocorticoid treatment

7. A GH response < 9 mu/l is indicative of severe GH deficiency. Values between 10-20 are borderline.

8. In Cushing's syndrome there will be a rise of < 170 nmol/l above the fluctuations of the basal cortisol whereas patients with depression show a normal cortisol rise with hypoglycemia.

9. Up to 20 % of patients with Cushing's syndrome show normal response to hypoglycemia.

Oral glucose tolerance test for acromegaly:

Indication:

When a clinical diagnosis of acromegaly is suspected.

Preparation:

Fasting from overnight.

Take blood sample for IGF1 , GH and glucose at 0 min

Administer 75 gms of oral glucose

Do the test supine (as dumping can cause faint producing GH rise)

Take bloods for GH and glucose at 0, 30, 60,90 and 120 min

Interpretation:

1. In normal individuals, the response is GH suppression to undetectable levels and atleast one of the values during the test should have undetectable levels.
2. In acromegaly, there is a failure to suppress GH to $< 2 \mu\text{l}$.and there may be a paradoxical rise in GH levels.

False positives:

Can occur in

- Chronic renal failure
- Chronic liver disease
- Malnutrition
- Poorly controlled diabetes
- Heroin addiction
- Adolescence(due to high pubertal GH surges)
- Osmotic dumping of high glucose load

Safe levels in Acromegaly:

Abundant epidemiological studies suggest that a GH level of 5 mu/l or less is associated with normal life expectancy. (recent studies suggest a value of <2 mu/l)

The most important determinant of outcome is the most recent GH or IGF 1 level.

The concept of safe hormone level rather than cure is realistic as normalisation of GH dynamics virtually never occurs in acromegalics.

A mean of several GH levels of 5 mu/l or less is equivalent to a nadir achieved during OGTT of <2 mu/l. Others measure average of GH at 30, 60, 90 minutes.

1 ng/ml = 2 mu/l

Prolonged Glucagon test:

Indication:

Assesment of GH and cortisol reserve especially when ITT is contraindicated.

Contraindications:

Phaeochromocytoma or insulinoma (may provoke an attack)

Severe hypocortisolemia or thyroxine deficiency.

Side effects:

Nausea is common and patients may rarely vomit.

Preparation:

Fasting from midnight

1 mg glucagon s/c, 1.5 mg if > 90 kg

Basal samples for glucose, cortisol and GH.

Further samples at 90, 120, 150, 180, 210 and 240 min

Values at 180, 210 and 240 min are especially relevant.

Interpretation:

Adequate cortisol response is defined as a rise > 170 nmol/l to a value > 550 nmol/l
GH rises by > 20 mu/l.

Note:

This is a less reliable test than ITT .

It is a good alternative in patients who have contra-indication to ITT.

The false negative rate for cortisol is around 30 % (but only 8% of normals will not show either a peak value of 550nmol/l or a rise of 170 nmol/l). Only 4-8% of normals will not show an adequate rise in GH, this is usually in patients over 50 years .

Severe deficiency of GH if response to glucagons is < 9 mu/l.

Adult Growth Hormone replacement:

Dynamic tests of GH secretion:

1. ITT is the most widely used test. Peak GH < 9 mu/l is diagnostic of severe GHD.
2. Alternative tests if ITT contraindicated includes Glucagon test, combination of GHRH and GH releasing peptide (GHRP) test, arginine stimulation test.
3. A second confirmatory test is required particularly in patients who have isolated GH deficiency.
4. A single dynamic test is sufficient to diagnose GHD in patients with 2 or 3 hormonal defects.

GHRH/GHRP test: (Ref JCEM VOL 85, No4, 2000)

Overnight fast.

GHRH 1 microgram/kg iv and GHRP 2, iv 0.1 microgram/kg both together at 0 min.

Bloods for GH at 0, 30, 45, 60, 90 min

Blood samples spun and stored at -20C

Interpretation:

Peak GH response > 17 mu/l

Arginine test:

Indication:

Second line test for GH reserve

Physiology:

Arginine leads to GH release

Contraindications:

None

Procedure:

Fast from midnight and administer 0.5 g/kg (max 30 g) arginine iv in 100 ml saline over 30 min from time 0. Sample glucose and GH at 0, 30, 60, 90 and 120 min

Interpretation: Normal response is a rise of GH > 20 mu/l

NICE guidelines on indications for GH treatment:

Recombinant human growth hormone (somatropin) treatment is recommended for the treatment of adults with growth hormone (GH) deficiency only if they fulfil all three of the following criteria.

- They have severe GH deficiency, defined as a peak GH response of less than 9 mU/litre (3 ng/ml) during the insulin tolerance test or a cross-validated GH threshold in an equivalent test.
- They have a perceived impairment of quality of life (QoL) as demonstrated by a reported score of at least 11 in the disease-specific 'QoL-assessment of growth hormone deficiency in adults' (QoL-AGHDA) questionnaire.
- They are already receiving full replacement with other deficient pituitary hormones as required.

Dose regimen:

GH therapy is started at low dose under supervision of endocrinologist.

Usual starting dose is 0.2 mg every night for 1 month increasing to 0.4 mg for the next month and so on. Usual final dose is 0.6-0.8 mg/ night

Senior Nurse in Area 6 will guide the patients regarding self administration of GH.

The dose is gradually increased till the IGF 1 is normalised to just within lower end of normal for age and sex range for the laboratory concerned. Others use standard deviation scores for age and sex to alter dosage.

Ideally monitoring at 6 months include a patient interview, QOL questionnaire, weight, blood pressure, IGF 1 levels. DEXA scan may be necessary at intervals.

A joint decision with the patient is taken as to continuation if clear improvement of these parameters has occurred but also taking into account the patient's wishes.

It would be useful to give patients a print out of information on Growth hormone treatment from our website: www.tayendoweb.co.uk

Contraindications:

- Active malignancy
- Benign intracranial hypertension (resolves on stopping treatment)
- Proliferative / Proliferative retinopathy in diabetes mellitus

A persistent or severe headache on GH treatment should be immediately reported to the Endocrine department and GH hormone discontinued until further evaluation.

Adverse effects:

Weight gain, Carpel tunnel syndrome

Arthralgia, Myalgia

Usually mild and self limiting and decrease with continuing therapy but may need dose reduction.

There are no data to suggest that GH therapy affects tumour development. (No evidence from long term studies in children of increased tumour recurrence with GH treatment. Insufficient long term data in adults)

GH treatment and pregnancy:

No long term data on effects of GH on pregnancy, but case reports do not suggest a detrimental effect on foetal outcome. However, until more data, GH should be stopped prior to pregnancy. Moreover as the placenta synthesizes GH variant, GH therapy may be unnecessary.

GH treatment and critical illness:

No good evidence for a beneficial effect of GH replacement during critical illness and many endocrinologists suggest discontinuation of GH treatment in severely ill patients.

Effects of GH replacement:

- Increase in lean body mass by 2-5.5 kg
- Mean reduction in fat mass by 4-6 kg
- Increased BMD at 12-24 months
- Improved exercise capacity
- Improved QOL and psychological well being
- Improved LV function and reduction in cholesterol

IGF-1 range (ug/L) used at Ninewells Hospital:

Male:	<u>20-40 yrs</u>	<u>>40 yrs</u>
	240-380	150-330

Female:	<u>20-40 yrs</u>	<u>>40 yrs</u>
	240-450	220-390

Water deprivation test:

Indication:

Used in the differential diagnosis of polyuria and thirst.

NB: a 24hr urine output of <2500mls and morning first pass urine osmolality >500mosm/l makes Diabetes Insipidus (DI) unlikely.

Contraindications:

Make sure other causes of polyuria have been excluded (glucose, K+, Ca, renal function)

Anterior pituitary hormone deficiency renders results meaningless as in particular, steroid and thyroxine deficiencies impair excretion of free water load.

Preparation:

Up to 8.30 am: Allow fluids overnight, light breakfast, no tea or coffee

9.30 –4.30 pm: No fluids, dry food permitted. Weigh hourly and contact team if weight loss is > 3% of initial weight

4.30 pm: DDAVP 2 mcg im and measure urine output hourly for 4 hrs .

Procedure:

[See attached CIU procedure](#)

Interpretation:

U4/ P4: Normal ≥ 1.8 , partial DI :1-1.8 , Complete DI <1

Diagnosis	After dehydration	After DDAVP
Normal	>750	>75
PP or partial CDI/NDI	300-750	<750
Cranial DI	<300	>750
Nephrogenic DI	<300	<300

If there is a partial response, this test does not reliably differentiate between PP and partial CDI or NDI.

If there is a partial response, discuss with Consultant regarding hypertonic saline test.

Hypertonic saline test:

Indications:

Only in special cases. To be authorised by Consultant

Assessment of possible mild diabetes insipidus and other subtle defects in vasopressin

Procedure:

Set up a cannula for infusion (500 mls of 5% saline) in one arm and a second cannula in the other for bloods.

Take blood for osmolality and vasopressin levels at –10, 0 min.

Then begin 5% saline at 4 ml/min ie 240 ml/hr

Take blood for Osmolality & Vasopressin at 30, 60, 90, 120 min.

Vasopressin requires 15 mls into lithium heparin chilled tubes kept on ice.

Then deliver the tubes immediately to clinical biochemistry as vasopressin is very unstable.

Record times precisely on the tube and forms.

Monitor BP and patient throughout the test.

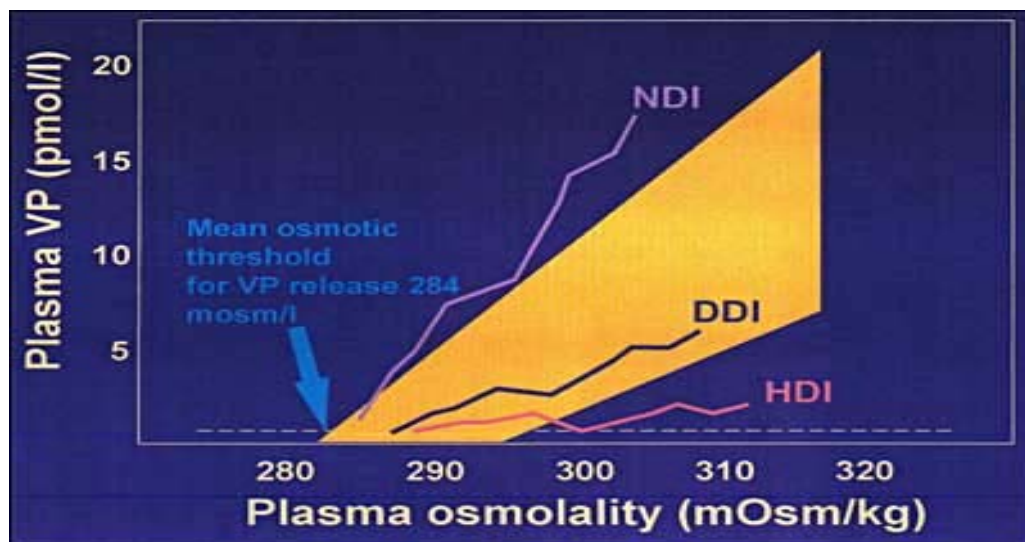
Note:

The biochemists have to be informed beforehand as they need to take precautions in the handling of the sample. It takes a few weeks for the results to be available.

Interpretation:

See nomogram

Differentiation of HDI from other forms of DI can be made by direct measurement of plasma VP during the controlled osmotic stress of a hypertonic 5% - sodium chloride infusion . Patients with HDI have undetectable VP levels during the progressive hyperosmolar stress, or values falling to the right of the normogram relating plasma VP to plasma osmolality .In NDI, plasma VP is inappropriately high for the prevailing osmolality, consistent with VP resistance. In DDI, the relationship of plasma VP to plasma osmolality is normal. Parallel assessment of the thirst response to hyperosmolar stress may show inappropriate thirst perception in this situation. Hypertonic stress testing is not interpretable if it produces significant nausea, as this acts as a powerful non-osmotic stimulus of VP release.



Acknowledgement

Ball SG and Baylis PH in Chapter 2 entitled 'Normal and abnormal physiology of the hypothalamus and posterior pituitary' on www.endotext.com

Cushing's syndrome:

Screening tests:

Urine 24 hr free cortisol (UFC):

High sensitivity (95%) but low specificity

False negative rate of 5-10%, false positive rate of 1-3%

9% of patients with Cushing's syndrome have at least one 24 hr urine collection within the normal range.

If several UFC's are normal, Cushing's syndrome is highly unlikely. However, measurement of cortisol/creatinine ratio may be more reliable.

Problems:

Raised 24 hr UFC's have been documented in 40% of patients with depression and 50% of patients with PCOS

Majority of problems associated with adequacy of collection by the patient.

Problem of cross reactivity becomes a particular issue if the possibility of exogenous glucocorticoid administration exists.

A low DHEAS because of suppressed plasma ACTH may be commonly found in and is a useful additional indicator of exogenous glucocorticoid administration.

Prednisone and prednisolone crossreact in most cortisol RIA while dexamethasone does not.

1 mg overnight Dexamethasone suppression test:

Indication

Initial screening test for Cushing's syndrome in a patient with a low clinical suspicion of Cushing's. If the index of suspicion is high, omit this test and go directly for the LDDST+ CRH Test

Contraindications:

Patients on enzyme inducing drugs e.g anticonvulsants may rapidly metabolise dexamethasone.

Oestrogens may induce CBG and artefactually increase total cortisol levels

Preparation:

Outpatient test with no particular preparation

Method:

9 am cortisol after 1 mg dexamethasone at 11 pm the previous night.

Interpretation:

Values of cortisol expected to suppress to <50 nmol/l. Definitely abnormal if cortisol > 100 nmol/l.

False positives will be higher at this cut off (50 nmol/l) but the main aim of this test is the ease of outpatient screening to exclude Cushing's

False negative rate is 2%, false positives in obese, alcoholics and hospitalised patients 10-20%

LDDST: (Low dose dexamethasone suppression test)

This test has a sensitivity and specificity of 98%.

Used to differentiate patients with Cushing's syndrome from normal individuals.

Procedure:

No particular patient preparation. Occasionally the test can be done as an outpatient procedure if you believe that the patient will take the tablets in time.

9 a.m cortisol and basal ACTH prior to the test

0.5 mg dexamethasone 6 hourly for 48 hrs . (09.00, 15.00, 21.00, 03.00) for 48 hrs

9 a.m cortisol at the end of the test (i.e. 6 hrs after last Dexamethasone dosage)

Contraindications:

Patients on enzyme inducing drugs, oestrogens

Care in diabetes mellitus and psychologically unstable patients

Interpretation:

This should lead to complete suppression of cortisol to < 50 nmol/l in normal subjects at 48 hrs, if the test is done precisely with dexamethasone given 6 hrly and last dose given 6 hrs before final blood sampling.

Conditions of non suppression of cortisol in the absence of Cushing's in LDDST

1. Alcoholic Pseudo Cushing's
2. Severe depression
3. Liver enzyme inducing drugs
4. Non compliance
5. Oestrogen therapy
6. Cross reactivity
7. Acute illness, infection.

Alcoholic Pseudo-cushing's

Biochemical abnormalities usually disappear within days of stopping drinking.

Admission of patient to investigation unit may allow closer observation, since sleeping cortisol value has been shown to be undetectable within 5 days of abstinence.

Depression:

The most common cause of pseudocushing's. A trial of antidepressants followed by retesting may be useful

Depressed patients may have an intact cortisol response to Insulin induced hypoglycemia whereas such a response is seen in only in about 80% of patients with Cushing's syndrome.

Dexamethasone Suppressed CRH Test:

Indications:

Confirmatory test for Cushing's

Contraindications:

As in LDDST

Method:

Dex 0.5 mg /6 hourly for 2 days followed by 1 microgram/kg of ovine CRH 2 hrs after last dose of dexamethasone.

Cortisol and ACTH done before first dose (0900). Dexamethasone written up at 6 hrly intervals (0900, 1500, 2100, 0300 hrs) for 48 hrs

Total of 8 doses written up till 3 am day 2.

The ninth dose should be written up after the Dex + 48 blood for cortisol is taken at 9 am on day 2.

It is important that patient must fast from midnight before CRH test, as food will affect dexamethasone absorption in the 2 hrs before the CRH test.

CRH test should follow **exactly** 2 hours after that dose of dexamethasone(0900 day 2), ie 11.00 a.m . This timing is very critical, as it will affect the 11.15 pm sample.

CRH given at dose of 1 mcg/kg or total of 100 mcg iv 11.00 am

Bloods taken at 11.00 am before CRH and at 11.15 am for cortisol

Interpretation:

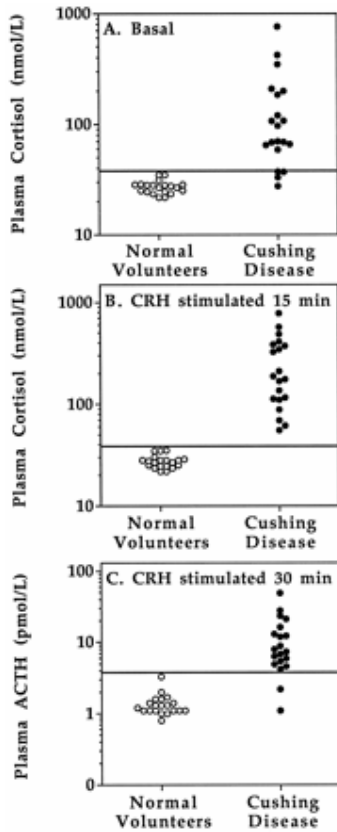
Dex + 48 hrs cortisol suppresses to < 50 nmol/l

A plasma cortisol value of > 38 nmol/l measured 15 minutes after CRH administration has 100 % diagnostic accuracy for Cushing's syndrome and has superior sensitivity and diagnostic accuracy when compared with either the low dose dex test or CRH test done alone.

The Dex Suppressed CRH test differentiates patients with Cushing's from pseudo-cushing's and normal subjects. (JCEM Vol 83,No2,1997).

However its value in patients with episodic hormonogenesis has not been tested.

There is no data yet on the value of 11.00 am sample day 2



Individual data showing the criteria with the best diagnostic accuracy. A, Basal plasma cortisol after dexamethasone-suppression; B, Dex-CRH plasma cortisol obtained 15 min after CRH stimulation; C, Dex-CRH plasma ACTH obtained 30 min after CRH stimulation; •, CD; ◊, healthy volunteers.

Basal testing for further evaluation of Cushing's:

1. ACTH:

Normal range: **9-52** ng/l

Extremely low or undetectable values usually indicates non ACTH dependant causes and one can directly proceed to adrenal imaging.

A few patients with pituitary dependant Cushing's may have ACTH levels in the low normal/low range.

In general plasma ACTH values in Cushing's disease tend to be lower than that of ectopic ACTH syndrome but overlap in 1/3 patients may prevent reliable discrimination.

2. Serum K+

K+ is almost uniformly low in ectopic ACTH secretion and this may be a very helpful discriminator.

Up to 10% of patients with Cushing's disease also exhibit hypokalemia.

Dynamic testing for further evaluation of Cushing's:

48 hr HDDST:

Can follow the LDDST. The final sample from LDDST can be taken as the basal sample for this test. This test can also follow Dexamethasone suppressed CRH test

During the test, patient takes 2 mg dexamethasone at strict 6 hr intervals (09.00, 15.00, 21.00, 03.00 hrs) for 48 hours

Cortisol is measured at baseline and 48 hrs later i.e. 6 hrs after last dexamethasone dosage.

Cautions:

1. Diabetes mellitus
2. Mood and psychiatric disturbances
3. Contraindications as in LDDST
4. Hypertension

Interpretation:

If the 0900 hr cortisol is less than 50 % of the basal value after 48 hrs of high dose dexamethasone, then this is suggestive of Cushing's disease (ie. pituitary origin). No suppression is suggestive of adrenal adenoma or ectopic Cushing's.

This test is useful but not totally reliable in the differential diagnosis of Cushing's as this is neither very sensitive nor very specific.

About 10 % of Cushing's disease do not suppress, 10% of ectopic ACTH tumours suppress (usually occult and relatively benign tumours with lower levels of ACTH and cortisol) and rarely adrenal tumours suppress.

Patients with ectopic tumours who suppress tend to have small benign tumours with lower levels of ACTH and cortisol, and these patients are very difficult to differentiate from Cushing's disease.

The criterion of 50 % suppression should not be applied too rigidly as many cases of Cushing's disease will suppress by 40-45 %. In difficult cases, it is advisable to repeat the test as no patients with adrenal tumour have been shown to have reproducible suppression and cases of Cushing's syndrome may show cyclical variation.

CRF test:

Basis:

In majority of patients with Cushing's disease, the intravenous administration of CRH causes an excessive rise in plasma ACTH and cortisol, while in patients with ectopic ACTH secretion such an effect is seen only very rarely.

Protocol:

Fast overnight

Insert venflon

Ensure patient lies supine for atleast 30 minutes prior to the test and remains so throughout the test.

1 microgram/kg of ovine CRF or total of 100 micrograms given IV and plasma ACTH and cortisol measured at -15,0,15,30,45,60,90 &120 minutes.

Test is well tolerated, the most common side effects being transient facial flushing in 20 % and rarely dyspnoea and hypotension.

Interpretation:

A rise in **cortisol** from basal to peak of > 20 % suggests a pituitary source

A rise of **ACTH** from basal to peak of > 50 % suggests a pituitary source

When criteria is used for ACTH responses, sensitivity is 86% and specificity is 95%.

When criteria is used for Cortisol, sensitivity is 91% and specificity is 95% (Meta analysis of 10 studies by Kaye and Crapo, Endocrine reviews)

Also, a rise by 35 % in ACTH at + 15 and +30 min (mean) in comparison to basal values suggest a pituitary source (Nieman et al JCEM 1993)

Problems:

10% of patients with Cushing's disease do not respond to CRH but they usually show suppression to HDDST.

10 % of ectopic ACTH syndrome may respond to CRH but these patients usually fail to suppress with dexamethasone

Thus HDDST and CRH test used in combination allows correct differentiation between pituitary and ectopic ACTH secretion in most cases.

Overnight 8 mg dexamethasone suppression test:

Basal cortisol at 8 am

8 mgs dexamethasone at bedtime

Cortisol at 8 am next day

Interpretation:

Suppression of plasma cortisol to < 50 % of the baseline indicates a diagnosis of Cushing's disease. The test has a sensitivity of 92% and specificity of 100%. Useful test to differentiate autonomous adrenal hyperplasia from pituitary source.

(Ann of Int Med 1986)

Inferior petrosal sinus sampling:

Indications:

1. To confirm or refute central / pituitary source of ACTH especially when the pituitary imaging is negative.
2. To attempt to lateralise the site of tumour prior to neurosurgical approaches.

Contact Consultant Endocrinologist if you think this test is necessary. This test is not done in Ninewells at the present moment and patients have to be referred to Glasgow for operator experience is essential.

Complications:

1. Discomfort in the ear
2. Catastrophic brain stem vascular damage (rare)
3. Thromboembolism

Contraindications:

1. Allergy to contrast dye
2. Orthopnea
3. Ischaemic heart disease
4. Bleeding tendencies

Procedure:

Metyrapone and ketoconazole to be stopped 1 week before the procedure

ACTH measured in all samples and cortisol in basal samples. CRH injected iv

Further details not elaborated as procedure not done at Ninewells

2 baseline samples at T -5 and T=0 minutes for ACTH and cortisol from each site (one each for IPS and one peripherally)

At T=0, CRF injected iv peripherally as bolus over 1 min

Simultaneous sampling of 3 sites for ACTH at T= 2, 5 and 10 minutes

Interpretation:

A basal ratio of >2 (central to peripheral) is consistent with Cushing's disease with a 95% sensitivity and 100 % specificity.

But more importantly, a peak stimulated central to peripheral ratio of ACTH of 3 or more is indicative of Cushing's disease.(this usually occurs between 3-5 min post CRH). The sensitivity is 100 %

If in addition, the basal or stimulated ACTH sample is 1.5 times (inter sinus gradient) higher than simultaneous contralateral side, this localises the pituitary adenoma with a sensitivity of 99% and specificity of 82%

False negative results occur in 4% whereas false positive results are extremely uncommon.

The success of this test is very much dependant on the operator.

Note:

Hypercortisolism should be demonstrated unequivocally on the DST prior to subjecting any patient to bilateral inferior petrosal sampling.

Imaging:

MRI pituitary:

MRI following gadolinium enhancement localises adenomas in upto 80% of cases.

Atleast 10 % of the normal population harbour microadenomas and therefore biochemical investigation is essential before interpreting results of imaging.

CT pituitary:

Hypodense lesion that fails to enhance with contrast.

Poor sensitivity of 47% and specificity of 74% (review of 9 studies of 278 patients with Cushing's disease).

Used when MRI is not possible.

CT Adrenals:

Mainstay of adrenal imaging.

MRI Adrenals:

Does not involve exposure to ionising radiation and hence preferred in children, young adults and pregnant patient.

It has a valuable role in characterisation of indeterminate adrenal lesion.

Assesment of cure for Cushing's syndrome:
Typical admission protocol

Monday

Admit. Cease hydrocortisone only.

Tuesday:

Cortisol 9 am, 12 noon, 3 pm, 6 pm, 9 pm
24 hr urine cortisol

Wednesday: (could be discharged at 10 am with instructions on dexamethasone and to come back to CIU at 9 am on Friday if adherence is likely to dexamethasone regimen otherwise remain as inpatient)

Dexamethasone 0.5 mg 6 hrly (9.00, 15.00, 21.00, 03.00)

Thursday:

Dexamethasone 0.5 mg 6 hrly (9.00, 15.00, 21.00, 03.00)

Friday:

9 am cortisol

Restart hydrocortisone after serum cortisol and review in clinic.

Interpretation:

Cure if

- Day cortisol profile: Mean of < 150 nmol/l
- LDDST, Cortisol < 50 nmol/l after 48 hrs
- Normal UFC ie <250 nmol/24 hrs and cortisol/creat ratio < 25
- Also good evidence if cortisol < 50 nmol/l pre morning dose then cure is likely.

Standard Short synacthen test:

Interpret results clinically and not in isolation.

Indication:

1. Diagnosis of adrenal insufficiency.
2. With basal ACTH measurements, useful in differentiating primary from secondary insufficiency.
3. In congenital adrenal hyperplasia.

Contra indication:

Anaphylactic reactions can rarely occur with patients who have a history of allergic disorders. Therefore better not done in asthmatics. Can use an alternative test eg glucagon /ITT in such patients.

Patient preparation:

1. If the patient is strongly suspected to have Addison's disease, treatment with dexamethasone should be started immediately and not delayed until the test has been performed, providing the test is performed within a short time of starting dexamethasone.
2. The patient should not be receiving ACTH, hydrocortisone or other steroids which would interfere with the assay. Those who are already on hydrocortisone should have their last dose 24 hrs prior to the start of the test.
3. No special preparation is necessary.

Procedure:

Synacthen is administered 250 micrograms i.m and cortisol measured at 0, 30, 60 minutes. When used to assess the ACTH reserve, only the 0 and 30 min values are required. The dose may also be given iv with equally identical results. Blood taken for ACTH and cortisol at 0 min, and cortisol at 30, 60 minutes.

Cortisol: clotted (gold topped tube).

ACTH: EDTA tube on ice and sent to lab immediately

Note: Patients should discontinue oestrogens replacement for 6 weeks before the test as increased CBG will make cortisol results difficult to interpret.

Intpretation:

Normal response:

- Basal cortisol in the reference range
- Cortisol increase above basal ≥ 170 nmol/l
- Peak cortisol > 530 nmol/l

Pituitary patients:

0 and 30 min cortisol is usually sufficient.

Basal cortisol > 450 nmol/l is highly suggestive of intact HPA axis.

If cortisol is < 350 nmol/l at 30 min then there is no need for ITT as this is indicative of deficient HPA.

If cortisol is > 600 nmol/l at 30 min, then ITT probably not required for this is indicative of intact HPA. (specificity of 96% Eur J endo 1998)

Using the 30 min cortisol value > 600 nmol/l provides a suitable substitute for ITT and this will decrease the number of ITT performed. (Clin Endo 1996).

Do ITT or alternative tests if cortisol at 30 min 350-600

Timing of the test:

Peak cortisol responses is unaffected by the time of the day. So this test can be done any time of the day. Preferably done in the morning as baseline ACTH gives a better clue as to the type of adrenal insufficiency, if present.

Peak Vs increment:

Increase in cortisol following ACTH is an unreliable index of adrenal function as it fails to distinguish normal patients from adrenal insufficiency. Smaller increases are obtained in the morning as endogenous ACTH levels and cortisol levels are already high. The peak cortisol response is unaffected by the time of the day and thus a more useful measure of adrenal function than the increment. (JCEM 1994)

Long synacthen test:

Indication:

Confirmation of diagnosis of adrenal insufficiency and to differentiate between primary and secondary adrenal insufficiency

(Note: Measurement of basal 0900 ACTH levels is far more sensitive than cortisol response in the long synacthen test)

Procedure:

0900: Insert cannula and flush
 Blood for cortisol and ACTH
 1 mg i.m depot synacthen (different from tetracosactrin for SST)

09.30, 10.00, 11.00, 13.00, 17.00, 09.00: Bloods for cortisol

Interpretation:

First 3 samples interpreted as in Short synacthen test

Serum cortisol fails to rise in primary adrenal failure whereas in secondary adrenal insufficiency, the cortisol levels gradually rise on post 10.00 hrs samples

This test is rarely used nowadays as it is time consuming and one gets a diagnosis based on sensitive basal ACTH assays.

Random Cortisol levels in acutely ill patients:(JCEM 1994)

Usually not very useful. If levels < 140 nmol/l , definite adrenal insufficiency.

If 140-360 nmol/l, presumptive adrenal insufficiency.

If 360-600 nmol/l, indeterminate test.

If > 600 nmol/l, probably normal.

Often indeterminate test. Therefore treat the patient irrespective of the value if high index of clinical suspicion.

Morning cortisol:

Often indeterminate.

Drawn between 6-8 am.

Production pulsatile, high levels in morning.

A value of < 100 nmol/l is very suggestive of adrenal insufficiency. (<85 nmol/l definite adrenal insufficiency according to JCEM article on lab diagnosis of adrenal insufficiency vol 79 ,4,1994)

ACTH levels:

Best test to separate primary from secondary adrenal insufficiency.

Not useful as diagnostic test.

ACTH production pulsatile and inhibited within few hours by exogenous glucocorticoids.

Must be drawn on ice and sent immediately on EDTA tube as unstable.

ITT:

Directly assesses HPA axis but note contraindications.

See ITT protocol.

Adrenal autoantibodies: (Ref CME review article, The endocrinologist Vol10, No1, 2000)

Adrenal autoantibodies are a good marker for subjects with ongoing autoimmune adrenal process.

The predictive value of ACA for clinical Addison's disease is high in children with hypoparathyroidism or type 1 diabetes mellitus.

In adults, the predictive value of ACA for clinical Addison's is lower than that observed in children.(20-30% vs 90%).

Several studies have shown that ACA can be found in 60-80% of patients with idiopathic Addison's disease.

There is a good correlation between ACA and 21(OH) Ab.

Prevalence of adrenal autoantibodies in other organ specific autoimmune diseases:

	ACA
Type 1 DM	0.2-0.9%
Hashimoto	1-1.5%
Grave's	1-1.5%
Premature ovarian failure	4-8.9%
Vitiligo	0.3-1%

NB: Consider adrenoleukodystrophy in adrenal ab negative patients and measure long chained fatty acids.

CRH test for adrenal insufficiency.

Procedure: See protocol as for CRH test in Cushing's

Interpretation:

The pattern of ACTH response to CRH allows the differentiation between primary, secondary and tertiary adrenal insufficiency.

3 distinct responses are apparent following CRH administration.

1. **Primary adrenal insufficiency:** High baseline ACTH levels which increases after CRH and declines slowly towards the baseline.
2. **Secondary adrenal insufficiency:** Low baseline ACTH levels which does not respond to CRH.
3. **Tertiary adrenal insufficiency:** Low baseline ACTH levels which show an exaggerated response to CRH and remains elevated for prolonged periods of time.

Problems:

The utility of CRH test as an initial diagnostic test has not yet been determined.

The distinction between primary and central hypoadrenalism can be made by less expensive tests.

The distinction from secondary and tertiary adrenal insufficiency is not therapeutically important.

Low dose synacthen test: (1 ug)

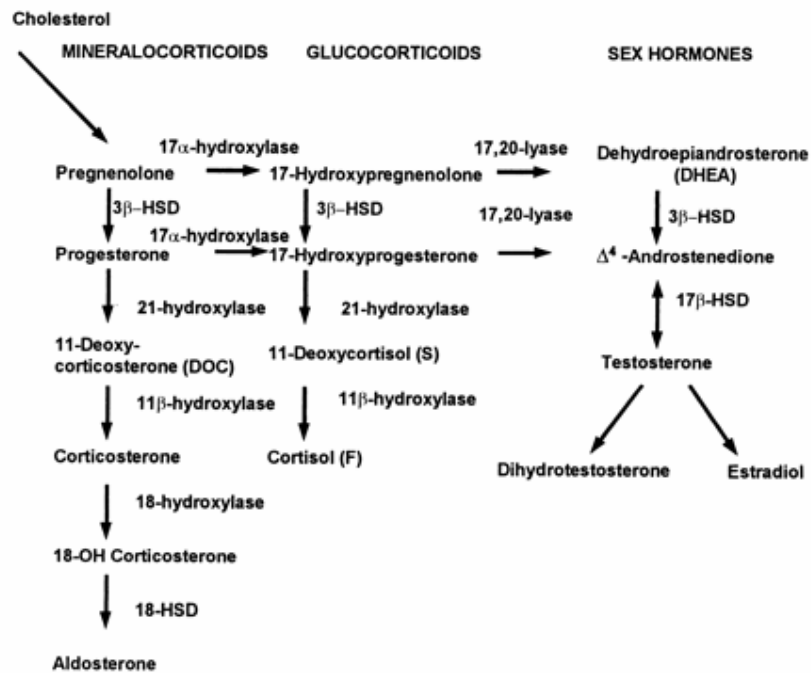
Much interest has arisen in the low dose synacthen test.

Proponents of low dose synacthen test argue that chronically understimulated adrenal glands may mount a satisfactory cortisol response to the unphysiological concentration of ACTH provided by 250ug of synacthen, but that only normal glands will respond to small doses used in this test.

The test is quick, a single sample is required at 30 min and can be performed any time of the day.

Concerns about the extent to which ACTH may be adsorbed to the plastic of syringes or saline bags dictates that further efforts at standardization and reproducibility of the low dose ACTH tests are required prior to its widespread recommendation for assessment of adrenal insufficiency.

Steroid hormones biosynthetic pathway:



Diagnosis of non-classic CAH.

Timing of measurement:

Screen in the follicular phase of menstrual cycle. 17 hydroxyprogesterone (17(OH) P) is produced by the corpus luteum, so false positive results may occur if the measured in the luteal phase of the cycle.

Must be measured at 9 a.m to avoid false negative results as 17(OH) P has diurnal variations similar to ACTH.

Normal range : upto 12 nmol/l

ACTH stimulation test:

Baseline 17(OH)P and 60 min following ACTH administration, 250mcg i.m

Exaggerated rise seen in non classic CAH.

As a thumb rule, 60 min value > 45 nmol/l is diagnostic of adult onset CAH.

Levels between 30-45 nmol/l suggest heterozygosity.

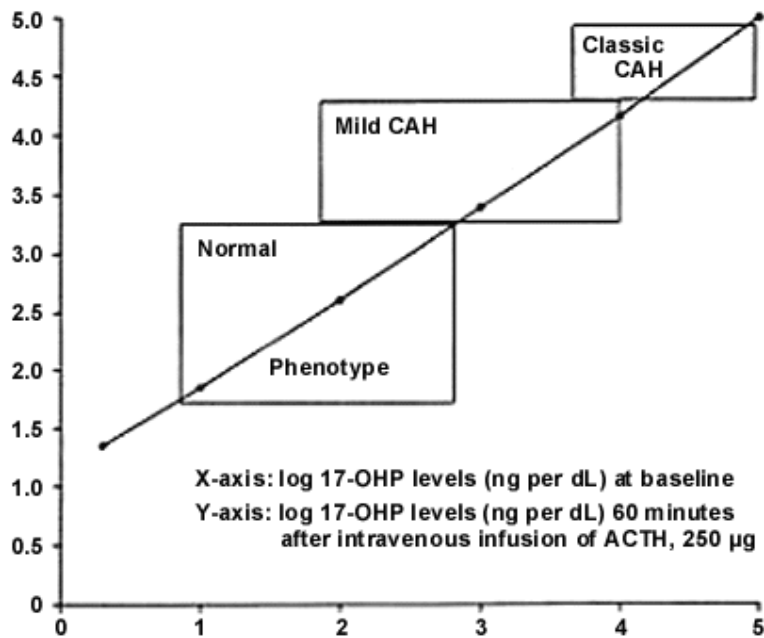
To convert ng/dl, nmol/L x 33.1 = ng/dl

Monitoring treatment:

Aim for a mildly elevated level of 17(OH)P (x2 normal). That would be < 20 nmol/l between 8-10 a.m in follicular phase.

Normalising 17(OH)P will result in complications from supraphysiological dose of steroids.

In pregnancy, use Androstenedione as 17(OH) P is naturally elevated.



Nomogram for analysis of 17-hydroxyprogesterone levels during an adrenocorticotropic hormone challenge in patients with suspected congenital adrenal hyperplasia. Note logarithmic scales. (CAH=congenital adrenal hyperplasia; OHP=hydroxyprogesterone; ACTH=adrenocorticotropic hormone). Data from references 2, 3 and 4.

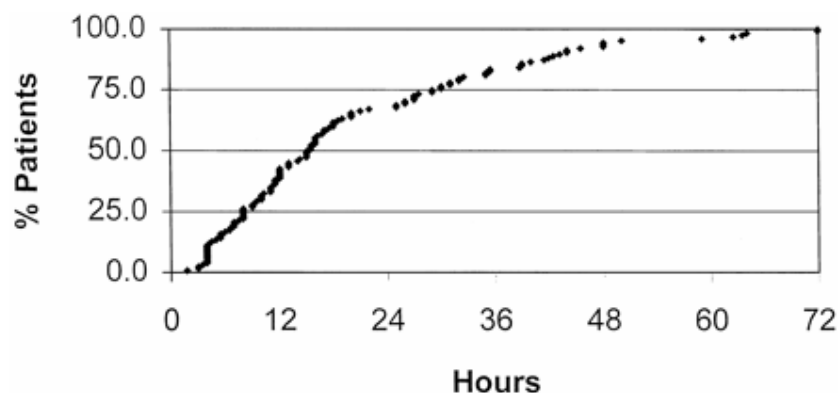
NOTE: Boxes as drawn are arbitrary delineations between various forms of the disease.

Protocol for the 48 hour fast test for insulinoma:

1. Date the onset of fast as of the last ingestion of calories.
2. Allow the patient to drink calorie free and caffeine free beverages.
3. Ensure that the patient is active during waking hours
4. Measure levels of plasma glucose, insulin, C peptide in the same specimen; repeat measurements every 12 hours. Do the above tests if patient symptomatic of hypoglycaemia anytime during the fast. Request Nurse to monitor BM's 6 hrly but send blood to lab for glucose measurement if ward glucose measurements <3.0 mmol/l.
5. End the fast when the plasma glucose is <2.2 mmol/l and the patient has signs and symptoms of hypoglycaemia. Do not reverse the hypoglycaemia until the lab confirms or unless the patient is unconscious and has fits.
6. At the end of the fast, measure the levels of plasma glucose, insulin, C peptide and sulphonylurea screen.(blood and spot urine sample)
7. If no symptoms during the fast, finish with 15-30 min exercise e.g a brisk walk around the hospital and remeasure.

48 hr vs 72 hr fast:

48 hour fast should replace the 72 hour fast test as in a series of 127 patients with insulinoma, the fast was terminated due to hypoglycemia in 42.5% in 12 hours, 66.9% by 24 hours and 94.5% in 48 hours. 7 patients fasted beyond 48 hours despite subtle neuroglycopenic symptoms and glucose and insulin concentrations were diagnostic of insulinoma. (JCEM Vol 85, No 9, 3222-6, 2000). See graph.



Interpretation:

1. Normals do not become hypoglycemic, although young women can run glucoses in the region of 2.2-3.0 mmol/l without symptoms.

2. True hypoglycemia **must** be demonstrated (glu<2.2 mmol/l) before interpretation of insulin results for the diagnosis of insulinoma.

Thumb rules:

- If hypoglycemia with raised insulin and low C peptide, consider self administration of insulin.
- If hypoglycemia with raised insulin and raised C peptide, make sure the sulphonylurea screen is negative.
- Insulin-glucose ratio is helpful in diagnosis of insulinoma

$$\frac{\text{Insulin (mU/L)}}{\text{Glucose (mmol/L)}}$$

Normal range: insulin/glucose <5

C Peptide Suppression test:

Principle:

Insulin administration will provoke suppression of endogenous insulin production which is assessed via C peptide assay.

Patients with insulinomas will generally fail to suppress endogenous insulin release as demonstrated by persistent inappropriate C peptide level after infusion or injection of insulin.

Indication:

Not routinely used

May be helpful in lending support to diagnosis of insulinoma in patients whose fast test is only borderline positive

Preparation:

- Obtain one or more fasting glucoses several days before the test, if any hypoglycemic results are obtained the reason for testing should be reviewed because of the risk of severe hypoglycemia.
- The patient should have consumed a high carbohydrate diet for three days prior to this test.
- The patient should fast from 10 pm on the night before the test.
- No smoking on the day of the test.

Precautions:

During this test it is possible that the subject may develop severe hypoglycemia or go into shock. The patient should be attended at all times to ensure severe hypoglycemia is detected and treated should it occur. This test is contraindicated in patients with heart disease or epilepsy.

Have the following at hand before the test:

- 50% intravenous dextrose injection
- 100 mg hydrocortisone
- 5% dextrose drip

Procedure:

Reference: Saddig et al, J of pancreas 2002: 3(1):16-25

Start the test in the morning.

2 iv canulae one on either arm.

Regular insulin (H.Actrapid) 0.075 U/kg/hr diluted with saline and infused at the rate of 15 ml/hr for 120 minutes.

If the fasting glucose is < 2.7 mmol/l, reduce insulin dose to 0.05 IU/kg/hr.

Blood samples taken at -15, 0, 20, 40, 60, 80, 100, 120, 135, 150 minutes to determine glucose and C peptide results.

If the glucose falls to < 2.2 mmol/l, blood should be taken for glucose and Cpeptide and the test terminated.

Interpretation:

C peptide usually suppresses and may be unmeasurable 30-60 minutes after hypoglycemia achieved. In insulinoma, C peptide is not suppressed with insulin administration. In a series of 16 patients with insulinoma, 15 out of 16 patients had a C peptidemia of over 400 pmol/l after insulin infusion (Service et al JCEM 1992)

Although not 100% reliable, this test can be completed in 2 hours and can act as a valuable screening or confirmatory test.

Localisation:

CT Abdomen:

Negative scan does not exclude an insulinoma. Reported detection rates vary from 20-40% to 60-70%

Somatostatin receptor scintigraphy:

Owing to the prevalence of somatostatin receptors on neuroendocrine tumours, SRS using indium pentetrotide theoretically allows detection of these tumours. Reported sensitivities are in the range of 53% in one study to 14% in another.

Other options are Preoperative transabdominal USS, endoscopic USS and intraoperative USS.

Screening for primary hyperaldosteronism:

Who?

- Patients resistant to two conventional antihypertensive medication
- Hypertension associated with hypokalemia
- Hypertension before the age of 50 years.

1. Serum K:

Hypokalemic alkalosis is highly suggestive but 20-40% may be normokalemic . A low salt diet will often mask hypokalemia.

2. Urinary K:

Excretion > 30 mmol/ 24 hrs following discontinuation of diuretics, in the presence of hypokalemia, is suggestive of hyperaldosteronism.

3. Aldosterone renin ratio:

Remember liquorice and carbenoxolone ingestion may mimic hyperaldosteronism.

K adequate

Discontinue drugs: (Ideally)

Spirolactone 6 weeks
Diuretics 4 weeks
ACE inhibitors 2 weeks
NSAIDS 2 weeks
Calcium antagonists 2 week
Sympathomimmetics 1 week
Beta blockers 1 week

This is often not ideal for screening, so often test is done with tablets(other than spironolactone).

Drug	Effect
ACE inhibitors and A2 AT	Increase PRA
Diuretics	Increase PRA
Spirolactone	Increase PRA
Beta blockers	Decrease PRA
Calcium channel antagonists	Decrease aldosterone

If anti hypertensive medication needed, Prazosin or doxazosin may be used.
A false negative is found in chronic renal failure.

Method:

Make sure hypokalemia is corrected first as low K will reduce aldosterone secretion

Sit patient quietly for 10 minutes.

Send Aldosterone (clotted) and rennin (EDTA on ice) to lab urgently.

Interpretation:

Calculate Aldosterone to Renin ratio

Abnormal if > 750 , > 2000 almost certainly Conn's, 750-2000 possibly Conn's.

The greater the ratio, the more the likely is the diagnosis of primary hyperaldosteronism.

SALT LOADING TEST;

Admit to CIU, remain of all usual outpatient medication as outlined before.

Date	Drugs	8 am bloods supine	12 noon bloods erect	Instructions
Monday	Give slow K 2 tabs twice daily throughout stay as IP			
Tuesday		Aldosterone, renin Cortisol Electrolytes	Aldosterone, renin Cortisol	Give slow Na 5 tabs at 18.00 hrs and 5 tabs at 22.00 hrs
Wednesday	Give slow Na 17 tabs/day	ie 4 at 8.00 5 at 12, 4 at 18.00 and 4 at 10pm	Fludrocortisone 0.5 mg at 8.00 am	
Thursday	Slow sodium and fludrocortisone as above			
Friday	Slow Na 4 tabs at 8 am, Fludrocortisone 0.5 mg at 8 am	Aldosterone, renin Electrolytes Cortisol	Aldosterone, renin Cortisol	Home afternoon.

Supine: lying in bed flat using 1 pillow for head support preferably from 12 midn but due to early morning toilet needs this may not be feasible, then min lying flat for 2 hrs before bloods.

Erect: Up and about preferably all morning but at least 30 mins, sitting in chair but not in bed.

Renin collected in EDTA bottle in ice. Aldosterone and cortisol in gold top.

Interpretation:

Non suppression of aldosterone ie > 140 pmol/l at 12.00 hrs upright or ambulant, and at 8.00 am supine, at the end of the test, is deemed diagnostic of primary hyperaldosteronism.

In bilateral adrenal hyperplasia, there is a $>33\%$ rise in aldosterone on rising while in adrenal adenoma, there is an anomalous fall in aldosterone.(see below on discriminative usefulness)

For the test to be valid, it is essential to measure the corresponding cortisol levels as in patients with APA, decline in circulating PAC occurs during the day corresponding to fall in ACTH and cortisol levels.

The postural study is based on the finding that circulating aldosterone levels in patients with APA show a diurnal variation due to sensitivity to ACTH and are relatively unaffected by changes in Angiotensin II levels whereas IHA is characterised by increased sensitivity to a change in angiotensin II levels that occur when standing.

The study is not fully discriminative of subtypes as it has been shown that some patients with APA are sensitive to angiotensin II and some patients with IHA are responsive to ACTH and show diurnal variation in aldosterone.

Once primary hyperaldosteronism confirmed, it is important to exclude the uncommon Glucocorticoid remediable aldosteronism

Strong suspicion if

- Patient with hypertension of early onset (<25 yrs of age)
- Prominent family history of death or morbidity from haemorrhagic stroke
- Refractory to conventional antihypertensives
- Tendency to become hypokalemic if diuretics used.

Dexamethasone suppression test:

Dexamethasone 1 mg orally every 12 hours 5 days.

Plasma aldosterone on day 5

Blood pressure measurements

Can be done along with adrenal scintiscan as radiolabelled cholesterol scanning (generally carried out after dex suppression to reduce the normal adrenal uptake of cholesterol) can be used to identify adrenal adenoma in patients with primary hyperaldosteronism.

Interpretation:

Plasma aldosterone levels are the endpoints in this test. The aldosterone falls to nearly undetectable levels in GRA and reflects the sole control of aldosterone by ACTH in this disorder. Blood pressure should improve after dexamethasone.

Pitfalls:

1. The duration of dexamethasone that should be employed is uncertain. Test length variability can cause misinterpretation of results as Dexamethasone suppression test (DST) of short duration (1-2 days) can result in false positives whereas longer duration gives false negatives.
2. DST during 5 days has been shown to be highly sensitive and specific for the diagnosing GRA.
3. GRA is not the only mineralocorticoid state that can be reversed with exogenous glucocorticoids. Syndromes like apparent mineralocorticoid effect and CAH have a salutary blood pressure response to glucocorticoids.
4. The other alternative for GRA is genetic testing for the chimeric gene which is highly sensitive and specific.

Selenium Cholesterol scanning for Conn's tumours:

Indication:

For functional lateralisation of Conn's tumours.

Caution:

Diabetes mellitus

Method:

Liase with Medical Physics in advance

Start Dexamethasone 0.5 mg qds at Day -2 of isotope injection

Isotope injection Day 0

Continue dexamethasone throughout the imaging procedure (usually 10 days)

Interpretation:

Conn's tumour should take up label with no uptake on the contralateral side.

Phaeochromocytoma:

Sensitivity and specificity of tests:

Plasma free metanephrines provide the best test for excluding or confirming phaeochromocytoma & should be the test of first choice. (JAMA March 20, 2002; 287:1427 Jacques W.Lenders). This assay is not routinely available.

Test	Sensitivity	Specificity
2x24 hr urinary free catecholamines	100 %	95%
Urinary metanephrines	80%	86%
Urinary VMA	65%	88%
Clonidine suppression test	97%	
MRI	98%	70%
CT	93%	70%
MIBG	80%	95%

False positives are rare in MIBG and about 10 % of tumours are not demonstrable (false negative). A small proportion of tumours do not take MIBG but yet take labelled octreotide.

Source(table) Oxford handbook of endocrinology.

Substances interfering with urinary catecholamines:

Increased catecholamines

Alpha blockers
B blockers
Levodopa
Decongestants
Metoclopramide
Domperidone
Hydralazine
Nicotine
Caffeine
Theophylline
Amphetamine

Decreased

MAO Inhibitors
Clonidine
Guanethidine

Variable effect

Levodopa
TCA
Phenothiazines
Ca channel blockers
ACE inhibitors
Bromocriptine

Note: Labetalol causes major interference in the estimation of plasma adrenaline by HPLC.

Clonidine suppression test:

Protocol:

1. The patient should be relaxed and comfortable in the bed.
2. IV cannulae
3. After 30 min (to allow the patient to settle after venepuncture) a baseline specimen for plasma catecholamines is collected.
4. Patient is given 0.3 mg of clonidine orally.
5. Blood for catecholamines is collected at 3 hrs.

Interpretation:

A decrease of more than 50% in plasma adrenaline and noradrenaline levels from the baseline levels.

Pitfalls:

False negative tests can occur in intermittently secreting tumours or those in whom the the norepinephrine levels are only marginally elevated.

False positive tests can occur in patients taking diuretics and TCA's.

Severe hypotensive episodes may occur during this test.

Pentolinium suppression test:

Indication:

To try and exclude phaeochromocytoma in patients with hypertension and borderline changes in plasma catecholamines or 24 hr urinary catecholamines

Contraindications:

No absolute contraindications but beware frail patient and patients with severe coronary or carotid vascular disease.

Side effects:

May cause severe transient hypotension.

Preparation:

Order pentolinium from Pharmacy. (availability can be difficult)

Stop hypotensive treatment for at least 24 hrs before the test (especially those centrally acting such as methyldopa)

Fast overnight

Quiet environment

Contact biochemistry beforehand.

Method:

Rest for 30minutes.

Patient lies supine

Baseline samples (x2 at -5, 0 min) for noradrenaline and adrenaline taken after 30 min.

At time 0 , give pentolinium 2.5 mg iv

Take blood samples at 60 minutes after pentolinium.

The patient remains supine 1 hour after the test to recover.

Interpretation:

In normal people, catecholamines suppress by 50 % whereas in Phaeos there is little or no suppression.

Calcium excretion rate:

Need spot urine and serum sample simultaneously.

$$\text{Ca E} = \frac{\text{Urine Ca (mmol/l)} \times \text{plasma creatinine } (\mu\text{mol/l})}{1000 \times \text{urine creatinine (mmol/l)}}$$

< 0.01 in Familial hypercalcaemic hypocalcuria (FHH)

>0.03 in Primary hyperparathyroidism (PHPT)

Pentagastrin test for Medullary Carcinoma of thyroid:

Indications:

Suspected medullary carcinoma of thyroid

Side effects:

Nausea and epigastric discomfort

Methods:

Patients should be fasted as food increases Calcitonin.

Check electrolytes and calcium

IV cannula

Baseline samples for Calcitonin

Give iv bolus of pentagastrin 0.5 mcg/kg body weight and flush cannula

Take samples at 2, 5 and 10 minutes for Calcitonin

Samples to be sent to lab on ice immediately (heparinised tube).

Interpretation:

An abnormal peak Calcitonin (> 200 ng/l) is suggestive of medullary carcinoma of thyroid.

Note: Many normals have been described with an exaggerated response to pentagastrin and the reproducibility of this test is poor.

Gastrinomas:

Once diagnosis is suspected, measure plasma gastrin after an overnight fast.

Sample must be placed on ice and sent to lab without delay.

Normal values up to 40 pmol/l

In gastrinomas, plasma gastrin levels are elevated, usually at 5-10 times the upper limit of normal.

Note: Proton Pump Inhibitors should be stopped at least 2 weeks prior to the test.

The next step is to measure gastric acid production to distinguish secondary hypergastrinaemia from primary hypergastrinaemia.

This procedure is adapted from the **Hammersmith Protocol**.

Preparation:

Liase with GI team with respect to equipments (autotitrator)

Stop H2 antagonists for 72 hours and PPI for 2 weeks

Stop antacids 24 hours before the test.

Patient should be fasting

Check autotitrator available, otherwise you will need a burette, conical flask, PH meter and 0.1 M NaOH

Method:

Pass the special double lumen NG tube with prior xylocaine spray to the nose and throat. Pass NG tube as far as the 50 cm mark at the nostril.

Ask the patient to drink 50 mls of water and then aspirate this via NG tube to check that it is in the most dependant part of the stomach.

Connect NG tube to the pump and collect 4 samples of gastric juice, each over 15 minutes into polystyrene cups. Alternatively, aspirate regularly and periodically with a 50 ml syringe to collect gastric juice over each 15 min period.

Measure total volume of each sample. Decant 10 mls of each into a fresh polystyrene cup and titrate against 0.1 M NaOH with automated titrating equipment.

Calculate the acid production in each 15 ml collection using the formula

$$A = (N/100) \times V$$

A= mmol of acid production

N= volume (ml) of 0.1 M NaOH needed to neutralise 10 mls of gastric juice

V= volume in mls of gastric juice in 15 min collection

A sum of the acid production for each 15 min will give the total hourly production

Interpretation:

Spontaneous basal acid output of 25-50 mmol/hr are diagnostic of gastrinomas, > 10 mmol/hr is suspicious. Post ulcer surgery > 5 mmol/hr is indicative

Hypergastrinemia and raised gastric acid are also found with

1. Gastric outlet obstruction, resolves with NG decompression
2. Massive small bowel resection, resolves few months post op
3. Antral G cell hyperplasia

Intravenous Secretin test:

Indication:

Strong clinical suspicion of gastrinoma with equivocal results on acid studies and fasting gastrin.

Inability to wean patients off antisecretory therapy for long enough to perform acid studies and gastrin estimation due to recurrence of severe symptoms.

Preparation:

Warn lab well in advance

Fast overnight. If possible, stop antisecretory therapy for 24 hours

Secretin ordered in advance from Pharmacy

Arrangements for transfer to lab on ice ASAP

Method:

Site iv cannula

2 baseline samples at -15, 0 minutes for gastrin

Secretin 2 u/kg injected as bolus at T=0

All samples sent on ice and assayed for gastrin

Interpretation:

In a study at NIH, a rise in plasma gastrin after secretin of 100 pmol/l (200 pg/ml) or above occurred in 87% of patients with gastrinomas with fasting gastrin concentrations between 50-400 pmol/l. A rise of 50 % over baseline value gives a sensitivity of 78%. Gastrin levels **fall** in normal individuals in response to secretin.

Urinary 5 HIAA:

Important to advise patient to **exclude** the following in the diet prior to collection:

Bananas

Pineapple

Walnuts

Chocolate

Coffee

Avocado

Salicylates

Chlorpromazine

L-Dopa

Alcohol

Vanilla essence (as in icecream)

I-123 and I 131 miBG

Used to determine the presence of pheochromocytoma and other neural crest tumours and their metastases.

The thyroid must be blocked when either I-123 or I-131 miBG are used for imaging organs unless such blocking is contraindicated on medical grounds.

For I-123 miBG imaging:

Potassium iodide 60 mgs tds, starting the day before the scan and continuing for 3 days

For therapy doses, the radiopharmacist should be consulted.

There is a large list of drugs that affect the uptake and retention of miBG, hence before requesting scan, consult with Nuclear Medicine staff to check if the patient's medication requires alteration.

Octreotide scan:

To locate a variety of tumours with excess somatostatin receptor binding sites, the most common of which is carcinoid.

Treatment with any somatostatin compounds must be discontinued for 1 month. Please contact Nuclear Medicine for advice.

Parathyroid scan:

Used to identify and localise solitary or multiple parathyroid adenomas

2 separate tracers are used in this study, one identifies thyroid and parathyroid and the other identifies the thyroid alone, in order to contrast uptakes.

Patient should stop thyroxine for 4 weeks prior to the test

There is a 2 hour delay between the administration of tracers and the patient being scanned.

The images take about 45 minutes to acquire.

Thyroid Scan : used to examine the function, structure and uptake of the thyroid gland. For thyroid scans the patient must have stopped taking thyroxine or T4 for 4 weeks prior to the scan. The patient's medication should be examined to check for the presence of large amounts of iodine. The patient must wait at least four weeks before having a thyroid scan if they have recently had a radiological procedure in which iodine was used, examples include an oral cholecystogram , a CT scan with iodinated intravenous contrast media.

The patient may continue taking beta blockers and carbimazole.

For more information contact Nuclear Medicine.

The patient receives an injection of radioactive tracer. After twenty to forty minutes some pictures are taken. The pictures take about half an hour. A calculation is done to determine the uptake of the tracer by the gland.

Wholebody I-131 scan : to identify residual thyroid uptake and presence of metastases in thyroid cancer.

The patient must stop taking carbimazole for 7 days prior to the scan.

For I131 treatment for thyrotoxicosis, cancer or whole body imaging, patients must take measures to avoid pregnancy for 6 months post administration.

REQUESTING A NUCLEAR MEDICINE INVESTIGATION

Before an investigation is booked it is the clinician's responsibility to:-

1. Ensure this procedure is appropriate and clinically necessary for the patient.
2. Check that the same investigation has not been carried out or requested recently by another clinician.
3. Document in the patient's notes that the test has been requested to avoid any duplication of request.

Patient preparation before investigation

1. If the patient is female, between the ages of 12 - 55, check that the patient is not pregnant or breast feeding at the time of booking. If this is the case, senior Nuclear Medicine staff should be contacted and notified of the situation.
2. The patient must be informed of the relevant preparation necessary before the investigation:- Outpatients will be sent instructions by post by Nuclear Medicine staff; inpatients should be told by clinicians requesting the investigation. For details of the patient preparation required, see notes on individual tests.

Advice to patient

1. An explanation should be given to the patient so that the patient understands why they are having the test, details including duration of investigation, any additional intervention that may be necessary i.e. blood sample etc., and the preparation required and precautions to be taken after the isotope.
2. For I-131 whole body imaging, patients must take measures to avoid pregnancy for 6 months.
3. Pregnancy must be avoided for 12 months after Se-75 selenocholesterol is administered to the patient for adrenal imaging.

Completion of request form

1. The request form should be filled in correctly and all questions answered to avoid any unnecessary delay in the investigation being carried out.

Incorrectly completed request forms will be returned to sender without a booking being made.

2. The Ionising Radiations (Medical Exposure) Regulations 2000 require Nuclear Medicine staff to ensure that the radiation dose to the patient is justified prior to administering a radioisotope. Thus, it is very important that appropriate clinical details are given so that Nuclear Medicine staff are able to make this determination.

Request forms without adequate clinical details will be returned to sender without a booking being made.

3. The correct name of the investigation should be clearly written on the request form or the appropriate box clearly ticked.
4. Ensure any additions on top of the basic procedure are clearly documented on the request form (to avoid repetition of the investigation at a later stage.) If you have any queries about this, contact nuclear medicine staff for advice.
5. If several investigations are being requested on one patient, each investigation must be requested using a separate request form (with the exception of a lung ventilation/perfusion study), however the Nuclear Medicine unit should be informed of the multiple requests so that there is an appropriate time interval between each investigation to avoid interference between studies.
6. The radioisotope injections given to children are reduced in proportion to the child's weight, hence, for patients under 18, the weight must be given in order that the correct dose for the child may be calculated.
7. The request form acts as a prescription for the administration of the radiopharmaceutical and hence must be signed by a medically qualified member of staff on the behalf of the consultant requesting the investigation.
8. In-patient requests should be telephoned to the department for priority booking.
9. It is the doctor's responsibility to make sure that the request form is in the Nuclear Medicine unit before the time of administration of the radiopharmaceutical otherwise the procedure will not be carried out.

Once the isotope is administered to the patient

The Clinician must be aware that once the isotope is administered to the patient, the patient can be considered as 'radioactive' and body fluids and vomit will be slightly radioactive. This must be remembered when doctors are arranging other investigations for the patient. Investigations which involve a long period of close contact with the patient, e.g. an ultrasound, should be arranged before the injection is given or on a different day to avoid unnecessary radiation dose to the staff involved. If blood or urine samples are sent to the laboratories, the laboratory staff should be informed that they may contain radioactivity

Perioperative preparation of thrototoxic patient:

- 1) Antithyroid drugs should be used preoperatively to achieve euthyroidism
- 2) Iodine compounds are not routinely necessary but can be used in patients who are intolerant of thionamides, require rapid preparation or have severe thyrotoxicosis needing multiple agents. Potassium iodide 60 mg tds 10 days prior to the procedure is used.
- 3) B blockers should be used unless contraindicated.
- 4) All patients should be as close as possible to clinical and biochemical euthyroidism prior to surgery as poorly controlled thyrotoxicosis has been associated with significant post operative mortality due to thyroid storm

Rapid preparation:

Rapid preparation is occasionally needed for patients requiring emergent or urgent surgery

The following regime is based on article from Endocrine and Metabolic clinics of North America (June 2003)

Propranolol 40-80 mgs tds po or esmolol 50-100 ug/kg/min

PTU 200 mgs po 4 hourly

Iopanoic acid 500 mg po bd

Hydrocortisone po or iv 8 hrly or Dexamethasone 2 mg po/ iv 6 hrly

B blockers to be continued post operatively, thionamides and iopanoic acid to be stopped immediately after surgery, steroids to be tapered over first 72 hrs

Complications of thyroid surgery:

- 1% recurrent laryngeal nerve palsy
- 1% permanent hypoparathyroidism
- 3% wound haematoma
- 5% wound infection
- Hypothyroidism

Hypocalcemia and thyroid surgery:

Incidence of temporary hypocalcemia reported between 6.9% to 25%

Manipulation of parathyroid glands producing transient parathyroid insufficiency is a commonly accepted mechanism .

Hypocalcemia that is associated with thyroidectomy for Grave's disease is often more severe and prolonged than for other surgical indications.

Patients who receive a total thyroidectomy, subtotal thyroidectomy, or complete thyroidectomy should be closely monitored for signs and symptoms of hypocalcemia.

Calcium and Mg levels should be checked 6-8 hrs in the immediate post op period.

Mild hypocalcemia should be treated with oral Calcium .

More severe hypocalcemia should be treated with calcium gluconate i.v followed by an infusion.

Parathyroid surgery:

Prior to surgery:

Pre op bloods including Ca, Phosphate, LFTS, Albumin, U&E

Ensure adequate hydration

After surgery:

Enquire for symptoms of hypocalcemia

Trousseau's and Chvostek's sign

Daily Ca (corr), U&E

If mild symptoms and Ca(corr) > 2 mmol/l, Sandocal

If severe symptoms and Ca (corr) < 2 mmol/l, for Calcium gluconate infusion

If persistently hypocalcemic, start alfacalcidol

Complications of parathyroid surgery:

Recurrent laryngeal nerve palsy

Wound haematoma

Wound infection

Transient or permanent hypocalcemia

DISCLAIMER

This guide is produced only for the use of medical staff in the endocrine section of Ninewells Hospital Dundee UK under the express direction, and interpretation of the Clinical Endocrine Specialists and should not be used by anyone else without permission of the specialist doctor in charge of the patient concerned, who should decide the most appropriate test to be performed on their patient taking into account all circumstances of each individual patient, local usage and experience. Variation in local laboratory analysis must also be taken into account in result interpretation if used by others. Please also note that the dosages illustrated (reflect UK version) should always be checked by those performing the test. This guide is not meant to include all tests available and should not be read as the most up to date interpretation of such tests as these are subject to on-going international research and experience in their usage.

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