EVALUATION OF ENDOCRINE FUNCTION

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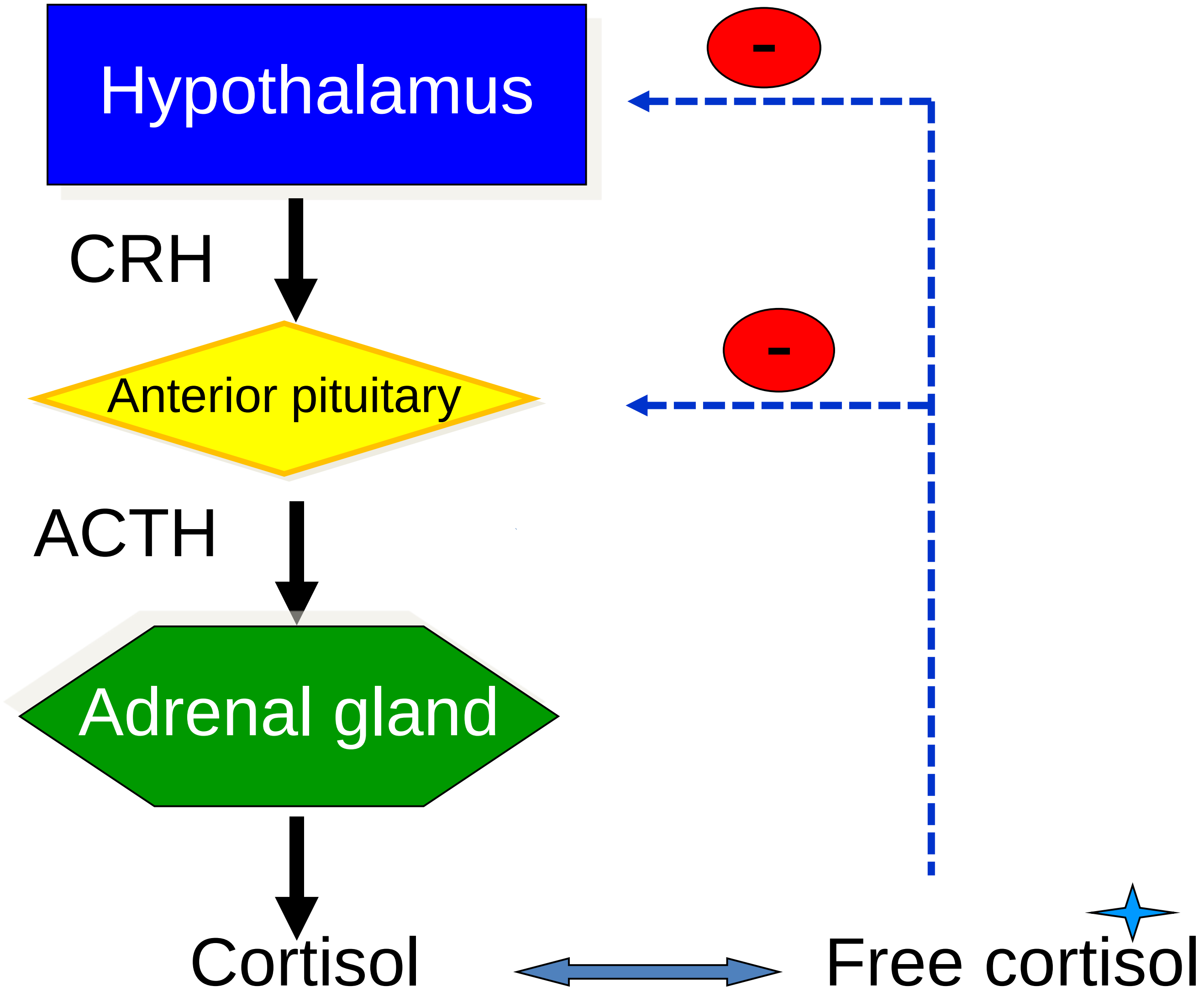
Objectives

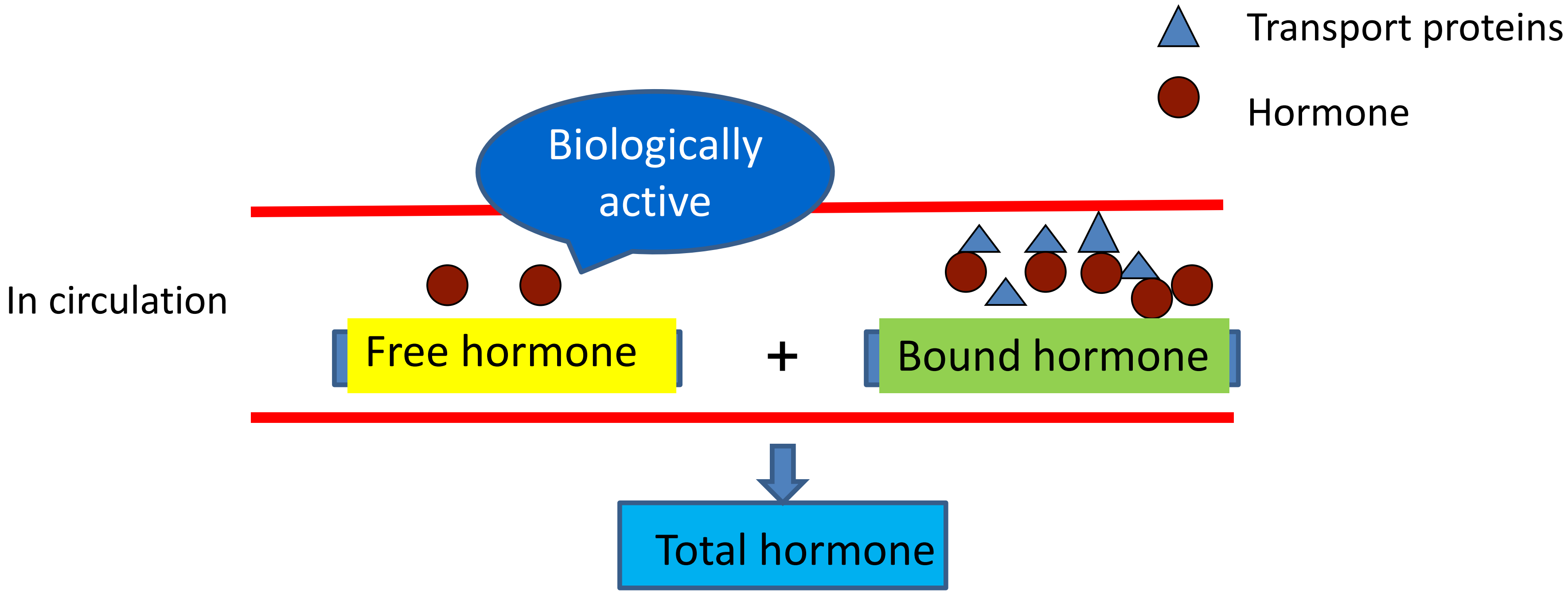
- สามารถประยุกต์ใช้หลักการควบคุมการทำงานของต่อมไร้ท่อในการแปลผลการตรวจทางห้องปฏิบัติการ และทราบปัจจัยที่ต้องระวังในการแปลผล
- ใช้การตรวจทางห้องปฏิบัติการเพื่อช่วยในการวินิจฉัยภาวะ Cushing’s syndrome

Hypothalamic-pituitary-adrenal axis



Considerable points for interpretation of cortisol result

- Plasma transport proteins



Considerable points for interpretation of cortisol result

- >90% of plasma cortisol is protein-bound

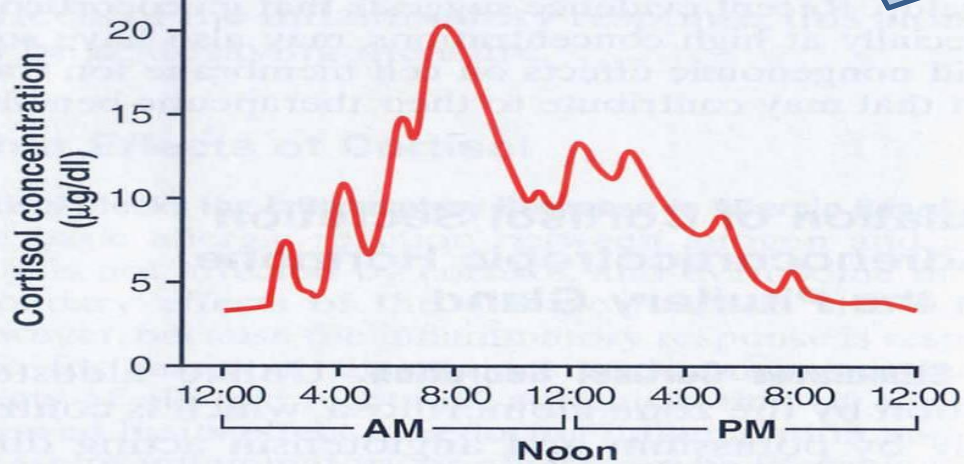
  : cortisol-binding globulin (80-90%)

  : albumin (10-20%)

Considerable points for interpretation of cortisol result

- Timing
  - Diurnal variation: Cortisol

ควรระบุ
เวลาเก็บสิ่งส่งตรวจ



Secretion of cortisol is highest in early morning and falls to a nadir at midnight

Figure 77–7

Typical pattern of cortisol concentration during the day. Note the oscillations in secretion as well as a daily secretory surge an hour or so after awaking in the morning.

Considerable points for interpretation of cortisol result

- **Stress** → acute increase in ACTH and cortisol secretion
  - Surgical trauma
  - Pyrogens
  - Hypoglycemia
  - Hemorrhage
  - Burn
  - Hypotension

Dynamic test of endocrine function

หากสงสัยภาวะ hypofunction

- Stimulation test
  - trophic hormone หรือสารกระตุ้นการหลั่งฮอร์โมน
  - Other conditions that stimulate hormone secretion

ไม่สามารถกระตุ้นให้หลั่งฮอร์โมนเพิ่มขึ้นได้

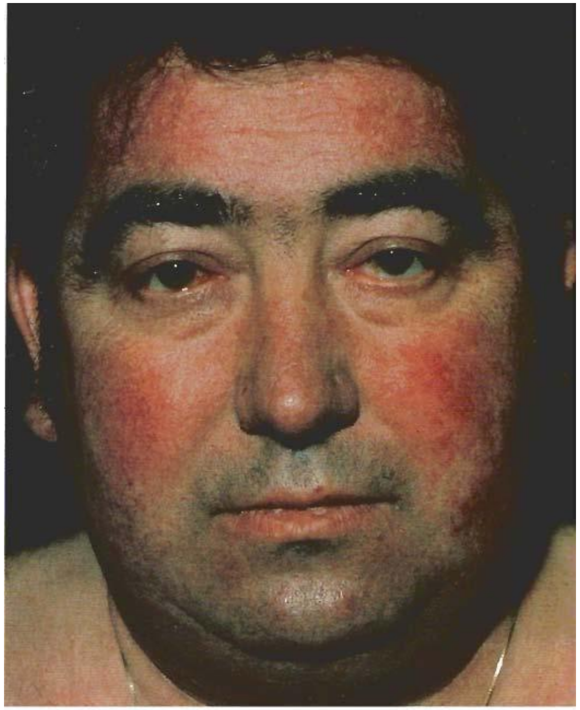
หากสงสัยภาวะ hyperfunction

- Suppression test
  - สารยับยั้งการหลั่งฮอร์โมน

ไม่สามารถยับยั้งให้หลั่งฮอร์โมนให้ลดลงได้

Laboratory Investigation for Cushing's syndrome

- Confirm the diagnosis
- Determine the cause



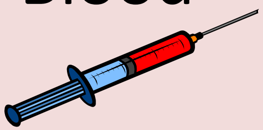
Laboratory investigation of Cushing's syndrome

Biochemical features of Cushing's syndrome	Test
• Hypercortisol • Loss of circadian rhythm of cortisol secretion	• cortisol measurement
• Disturbed feedback of the HPA axis	• low-dose dexamethasone suppression test (LDDST)

Cortisol Measurements

Specimen requirement

Blood



- serum or heparinized plasma cortisol

  เป็นการตรวจ total cortisol (free form + bound form)

Urine

- Urinary free cortisol
- 24-hour urine
- refrigerated at 2-8 ^{o}C

Saliva

- free cortisol
- refrigerated at 2-8 ^{o}C

Cortisol Measurements

	Advantages	Disdvantages
Blood	• convenience : specimen : method	• circadian variation • influenced by CBG ↑ estrogen concentration Stop for 6 weeks before test : Estrogen therapy : Pregnancy
Urine	• not influenced by circadian variation and CBG	• inconvenience :specimen collection :method • renal disease

Cortisol Measurements

Saliva

Advantages	Disdvantages
• convenience : specimen : not influenced by CBG	• specimen container • Salivette tube • circadian variation

Cortisol Measurements

Limitation

- Antibodies used in the assay show a high cross-reactivity with other steroids.

- Cortisol is increased in patients with hypoglycemia, stress, pregnancy, alcoholism, endogenous depression.

Laboratory investigation of Cushing's syndrome : **Plasma cortisol**

- Loss of circadian rhythm
- Midnight plasma cortisol level

>7.5 μg/dL

Measurement of salivary cortisol

- Disadvantage
  - Admit in the hospital for 24-48 hours
- False positive
  - Stress of venipuncture
  - Illness, admission in the hospital

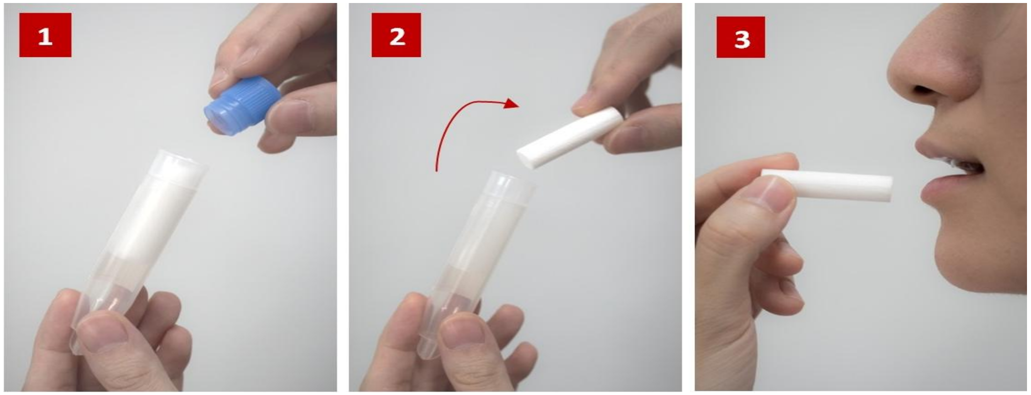
Laboratory investigation of Cushing's syndrome : **Salivary cortisol**

- CBG is absent in saliva → free cortisol
- Late-night salivary cortisol level (2 collections)

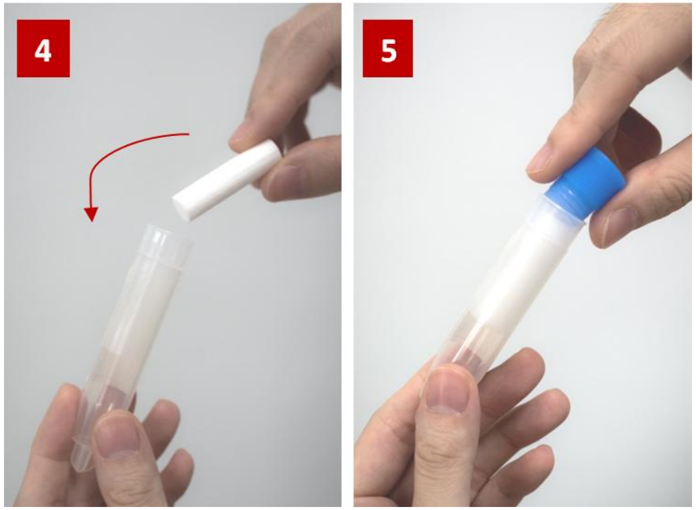
- Advantage
  - Not require hospitalization
- Note
  - Saliva sample on two separate evenings between 23.00-24.00 h
  - Avoid cigarette smoking on the day of collection or more
  - Avoid contamination from steroid-containing lotion or oral gels

Salivary cortisol:

Specimen collection use Salivette tube



เคี้ยวสำลีนาน 2 นาที



เก็บในตู้เย็นที่อุณหภูมิ 2-8 ^{o}c พร้อมส่งตรวจ

บันทึกชื่อ สกุล วันที่และเวลาที่เก็บน้ำลาย

Laboratory investigation of Cushing's syndrome : **Urinary free cortisol (UFC)**

- UFC concentration

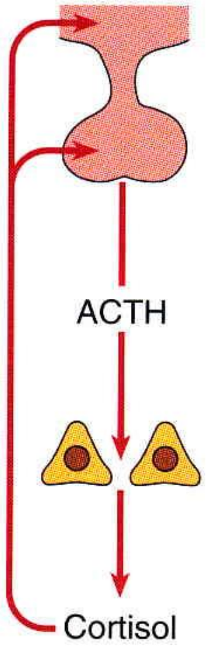
  > upper reference range at least 2 collections

  (4 times the upper reference limit are diagnostic for Cushing's syndrome)

- Note
  - UFC value can be normal in up to 8% to 15% of patients with Cushing's syndrome.
  - Moderate elevate results should be verified by further test.

Laboratory investigation of Cushing's syndrome: **Low-dose dexamethasone suppression test**

Principle



- Normal subjects results in suppression of HPA axis after administration of exogenous glucocorticoid.

- Dexamethasone
  - Potent glucocorticoid
  - Does not cross-react with cortisol assay.

Laboratory investigation of Cushing's syndrome
: **Low-dose dexamethasone s**

Useful outpatient screening test

- 1-mg overnight dexamethasone suppression test(DST)
  - 1 mg dexamethasone 23.00-24.00
    →Plasma cortisol at 8-9 am the following morning
  - Cut-off 1.8 µg/dL (sensitivity > 95%)

- Longer low-dose DST (2 mg/d for 48 h)
  - 0.5 mg dexamethasone begin at 9.00, 15.00,21.00 and 3.00 h
    →Plasma cortisol is measured at 9 am (Day 0), 48 h later (6 h after last dose)

Laboratory investigation of Cushing's syndrome : **Low-dose dexamethasone suppression test**

- Normal
  - Negative test = suppression of cortisol secretion

- Cushing's syndrome
  - Positive test = no suppression of cortisol secretion

False positive

Plasma dexamethasone level

Dexamethasone is rapidly metabolized: Drugs: phenytoins, phenobarbital, carbamazepine, rifampicin

ผู้ป่วยที่สงสัย
Cushing's syndrome

↓

Rule out Exogenous Cushing's syndrome

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Endogenous Cushing's syndrome

Laboratory investigation

- ***1st step: rule out Exogenous Cushing's syndrome***
  - History of exogenous glucocorticoid exposure

    ⬇

    HPA axis suppression

    ⬇

    central adrenal insufficiency

  - Laboratory investigation

    Plasma/serum cortisol at 8.00-9.00 a.m. *(after stop steroid drugs for 3-5 days)* <3µg/dL is indicative of adrenal insufficiency.

Laboratory investigation

- ***1st step: rule out Exogenous Cushing's syndrome***
- Laboratory investigation
  - Normal cortisol level during stress does not exclude adrenal insufficiency. If suspected→ ACTH stimulation test
  - However, a random cortisol of > 25 µg/dl during stress → unlikely adrenal insufficiency → unnecessary to perform an ACTH stimulation test in critically ill patients

Laboratory diagnosis
of
Endogenous Cushing's syndrome

2nd step: determine endogenous Cushing's syndrome

Three screening tests:

- 24-h urinary free cortisol
- 11pm-midnight salivary cortisol (late-night salivary cortisol)
- 1-mg overnight DST

Perform one of following tests then confirm by the other test

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Both tests are abnormal

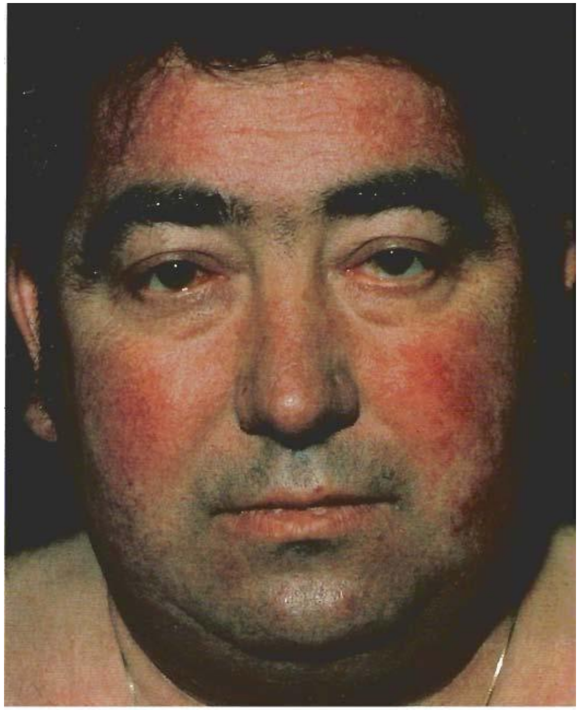
⇓

cause

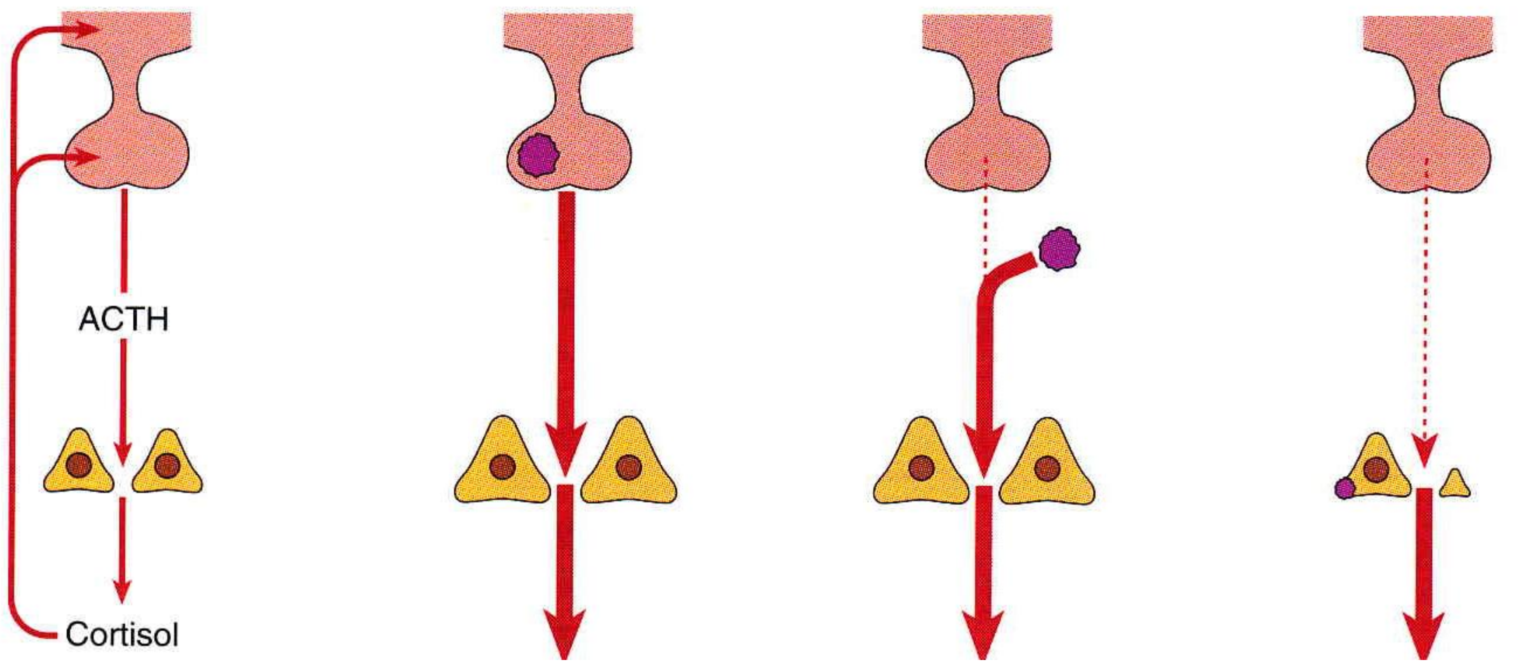
Laboratory Investigation in Cushing's syndrome

- Confirm the diagnosis

- Determine the cause



Cause of Cushing's syndrome



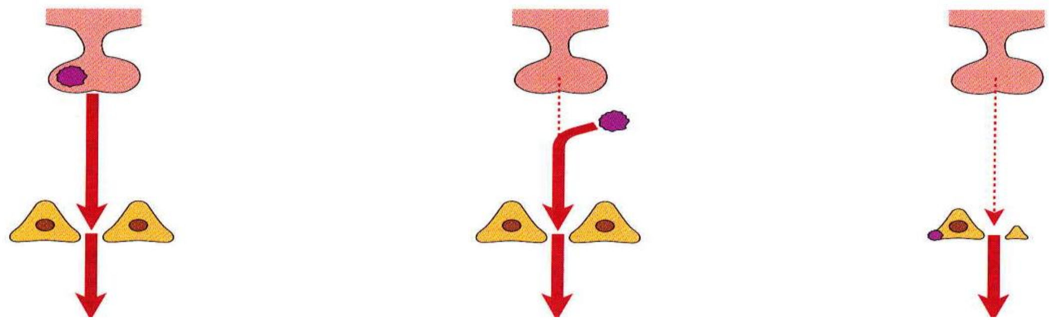
Normal

Pituitary cause

Ectopic ACTH

Adrenal cause

Cause of Cushing's syndrome



	Pituitary cause	Ectopic ACTH	Adrenal cause
ACTH	High (up to 50% normal)	High (often higher)	Low
	ACTH dependent CS		ACTH independent CS

ACTH Measurement

- Specimen
  - Plasma : plastic tube containing EDTA
  - should not stored in glass because ACTH becomes adsorbed to glass.

- Limitations
  - Instability→specimen should be kept in ice bath
  - If plasma cannot be analyzed immediately it should be stored below -20^{o} C.

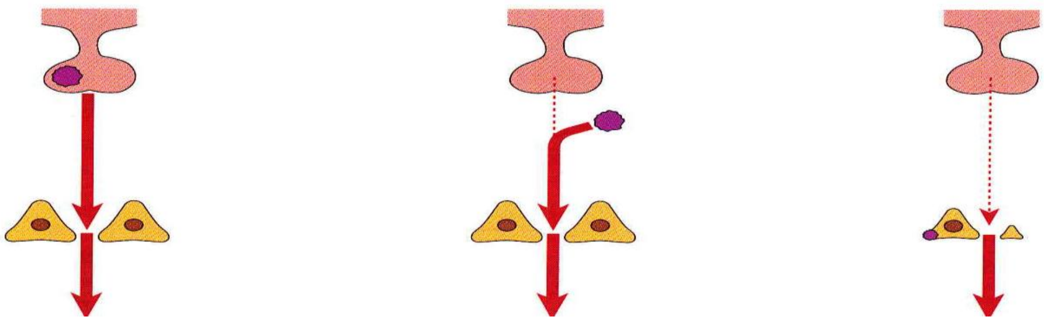
Dexamethasone suppression test for determine the cause of Cushing's syndrome

1. 8-mg overnight dexamethasone suppression test
   - 8 a.m. plasma cortisol is obtained then
   - →8 mg dexamethasone orally at 11 p.m.
   - →8-a.m. plasma cortisol is obtained the next morning

2. High-dose dexamethasone suppression test (HDDST)
   - 2 mg dexamethasone orally begin at 9.00, 15.00,21.00 and 3.00 h for 2 days

plasma cortisol or urinary free cortisol
After the test compared with baseline >50% reduction

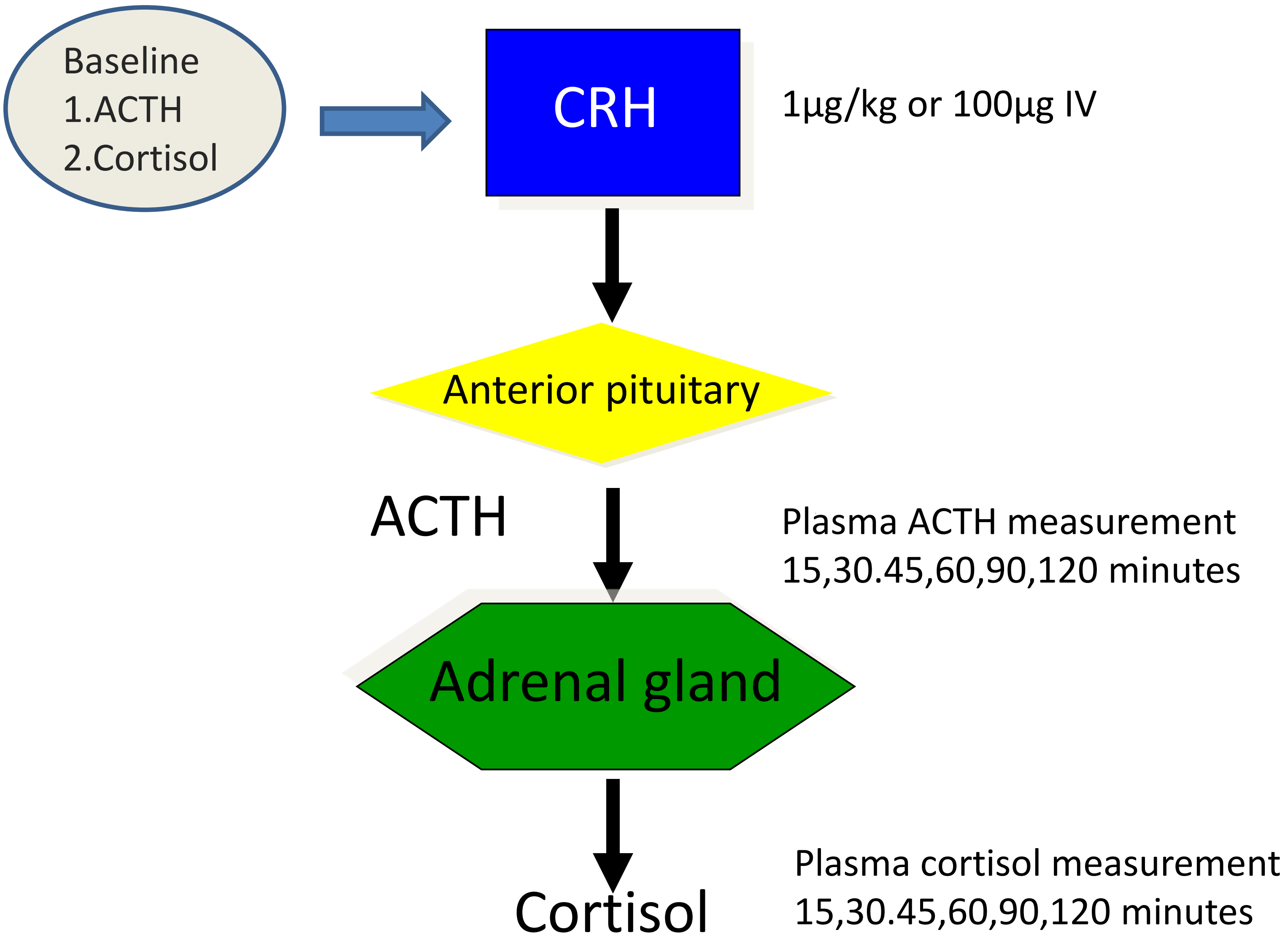
Interpretation of ACTH and high-dose dexamethasone suppression test



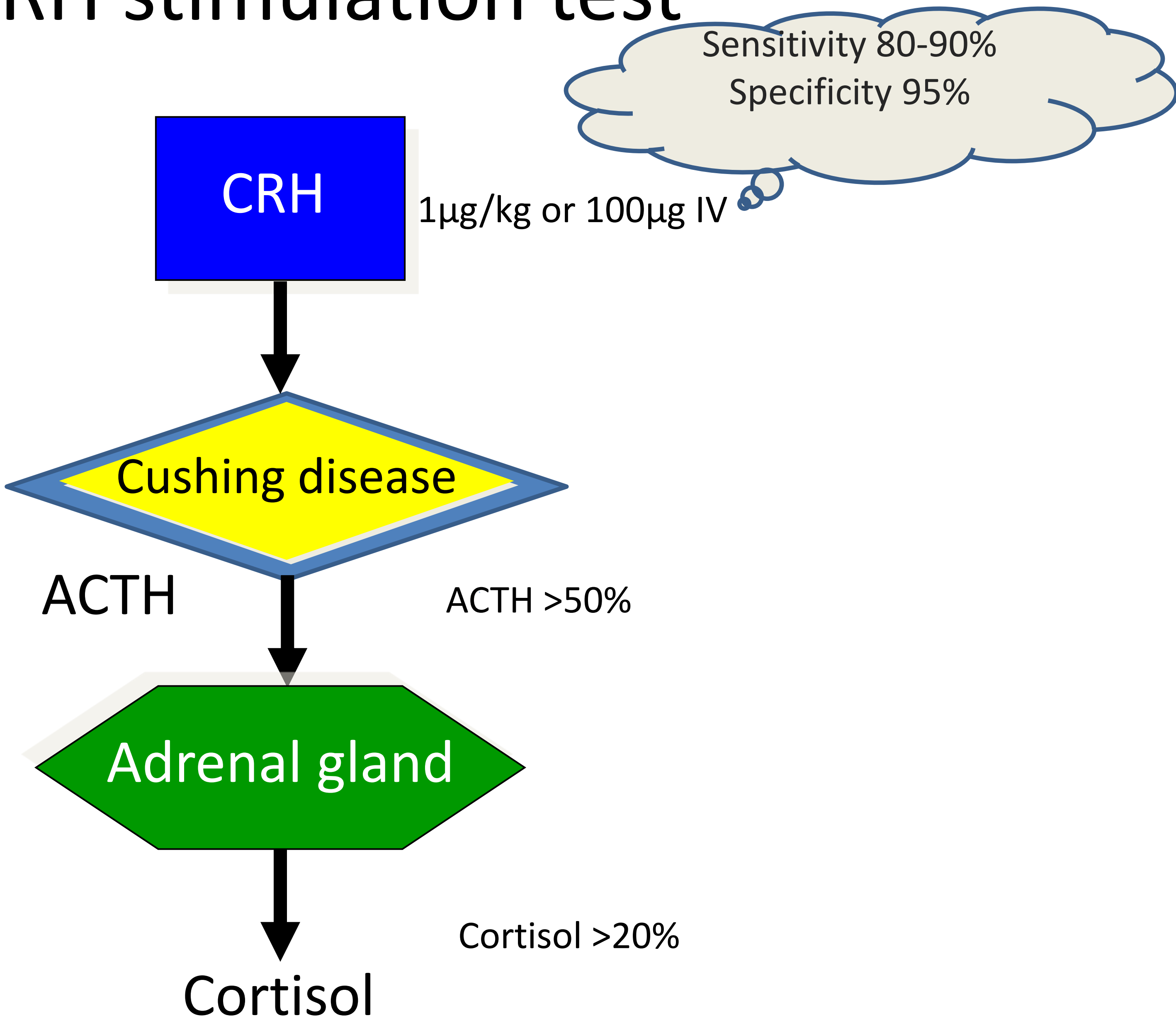
	Pituitary cause	Ectopic ACTH	Adrenal cause
ACTH	High (up to 50% normal)	High (often higher)	Low
High-dose DST	Suppressed (≥ 50%)	Not suppressed (<50%)	Not suppressed (<50%)

High-dose DST : sensitivity 60-90%, specificity 60-90%

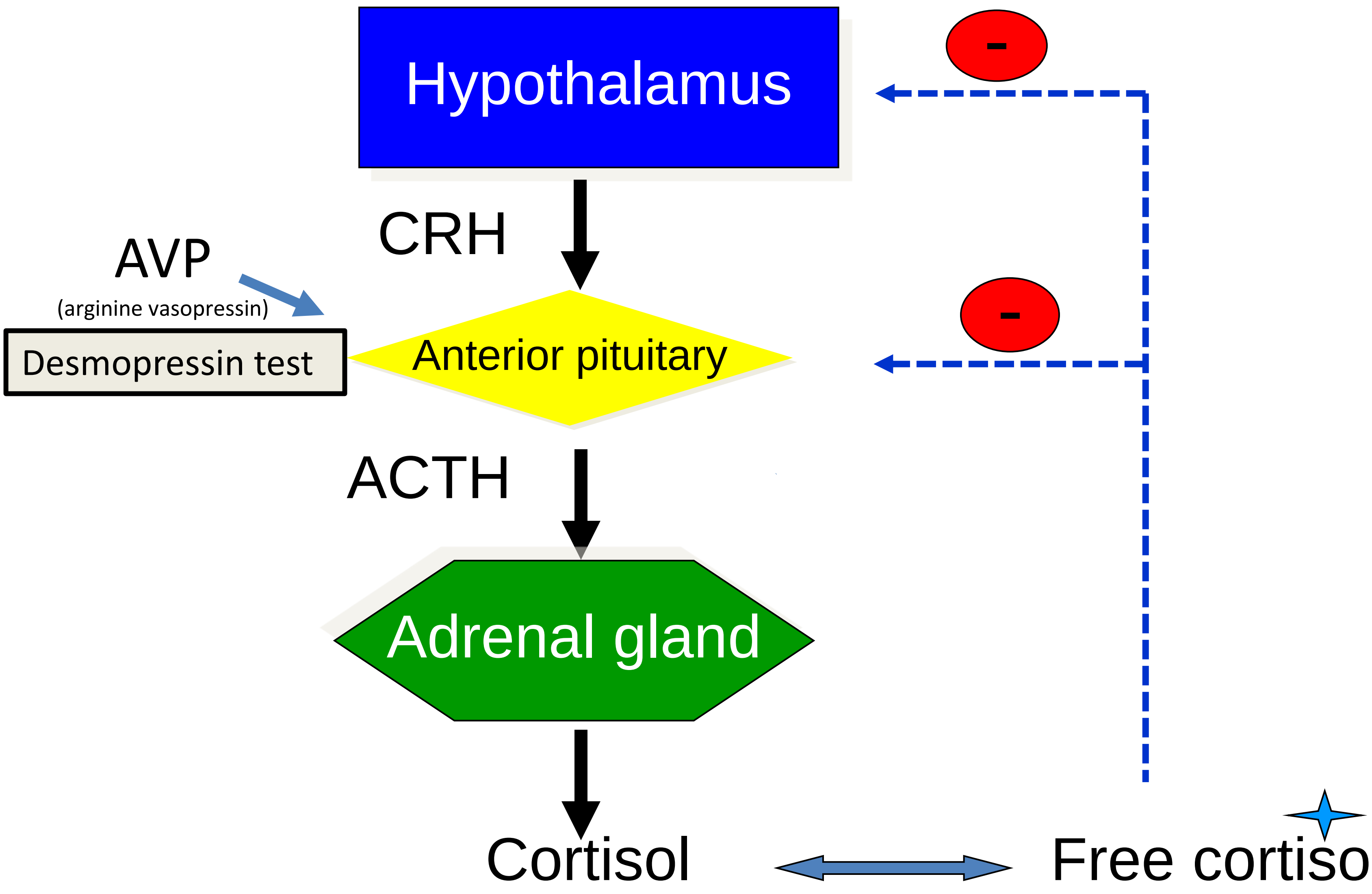
CRH stimulation test



CRH stimulation test



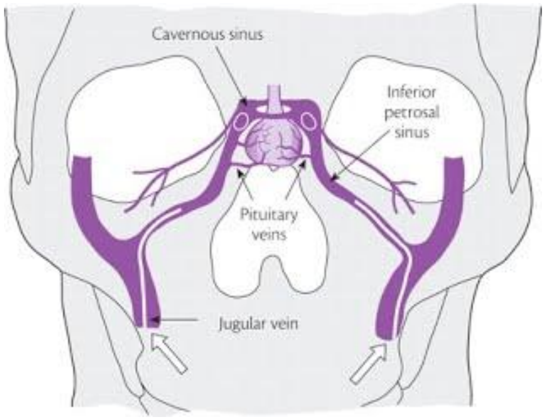
Hypothalamic-pituitary-adrenal axis



Bilateral inferior petrosal sinus sampling

- The most reliable assay for distinguishing pituitary and ectopic sources of ACTH hypersecretion
- ACTH
  - Central ACTH(IPS)
  - Peripheral ACTH(plasma)

Invasive,high cost complication



Cushing's disease

Central /peripheral ACTH >2 → **CRH IV** → Central /peripheral ACTH >3

CONCLUSION

Confirm the diagnosis
of Cushing’s syndrome

- **Laboratory investigation for endogenous Cushing’s syndrome**
  - *Urinary free cortisol (at least two measurements)*
  - *Late-night salivary cortisol (two measurements)*
  - *1-mg overnight dexamethasone suppression test (DST)*

Determine the cause
of Cushing’s syndrome

  - ACTH
  - High-dose dexamethasone suppression test
  - CRH stimulation test
  - Bilateral inferior petrosal sinus sampling

RATIONAL LAB USE

นึกถึงพยาธิวิทยาคลินิก

Plasma Cortisol :

define timing of collection, stress,
aware of steroid drugs that cross-react with assay

Salivary cortisol :

11 pm-midnight salivary cortisol
The patient can collect sample at home.
Define time and date of collection at tube

RATIONAL LAB USE

นึกถึงพยาธิวิทยาคลินิก

Random plasma cortisol during stress > 25 μg/dL

unlikely adrenal insufficiency

↓

Unnecessary to perform an ACTH stimulation test