Vcheck c.Cortisol

Mia Park

Overseas Dep.

14.10.2020

BIONOTE

Vcheck c.Cortisol

01 What is Cortisol?

02 Cushing’s disease

03 Addison’s disease

04 Product introduction

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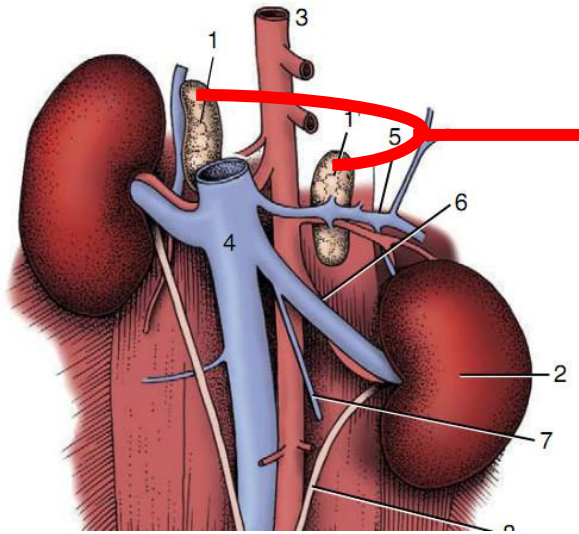
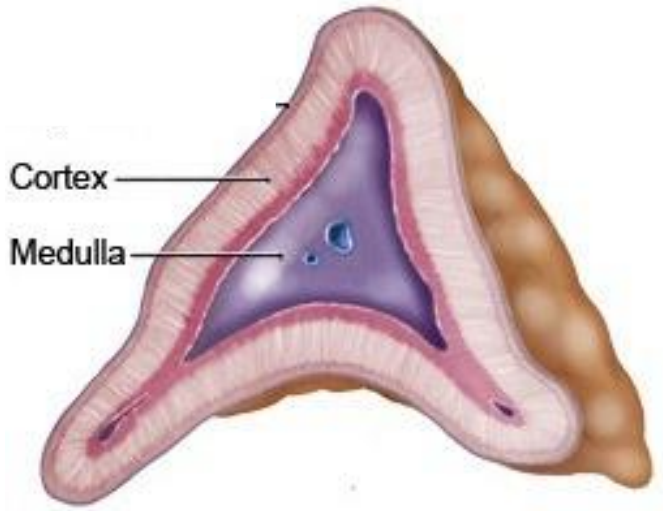
01 What is Cortisol?

Adrenal Glands





- ✓ Adrenal glands: Small endocrine glands located forward of the kidneys
- ✓ Function: Regulate hormone balance & metabolism

- ✓ Cortex: Steroid hormones
- ✓ Medulla: Adrenaline and noradrenaline

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Dyce et al. *Textbook of Veterinary Anatomy-E-Book*. Elsevier Health Sciences, 2009.

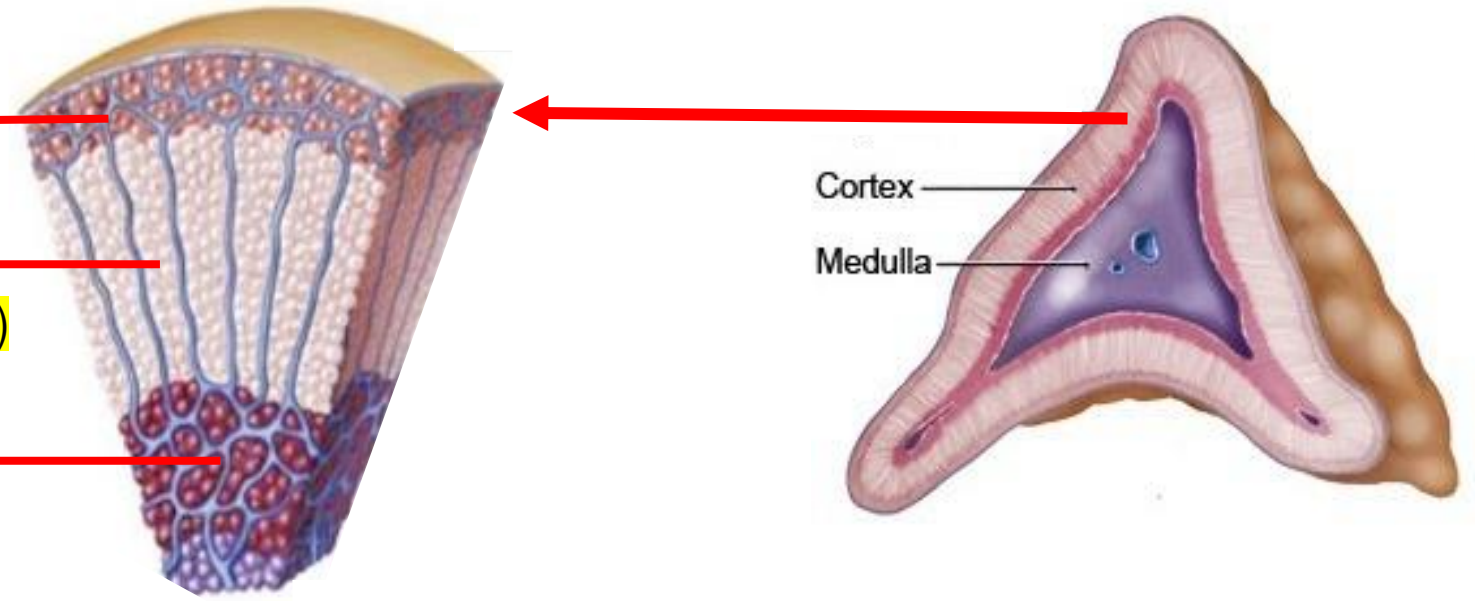
01 What is Cortisol?

Adrenal Cortex

Zona glomerulosa
→ Mineralocorticoid

Zona fasciculata
→ Glucocorticoid (e.g. Cortisol)

Zona reticularis
→ Androgen



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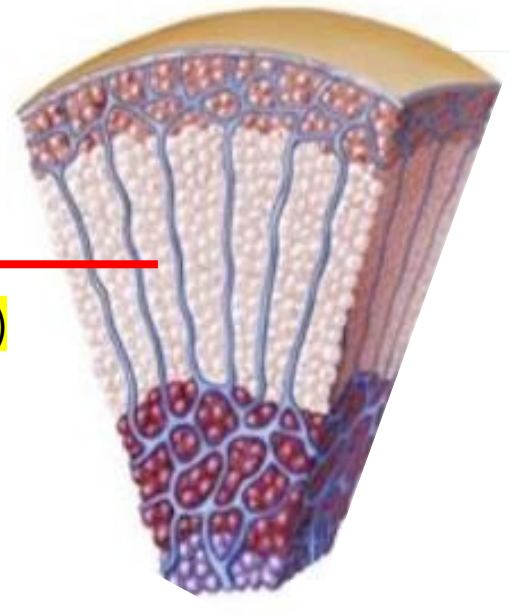
01 What is Cortisol?

Glucocorticoid Effects

Zona fasciculata

→ Glucocorticoid (e.g. Cortisol)

- ✓ Immune system
- ✓ Metabolism



Physiological	Pharmacological
Tempers immune and inflammatory processes	Anti-inflammatory Immunosuppression
Gluconeogenesis	Hyperglycemia
Potentiate Catecholamines	Hypertension
Increases RBCs	Erythrocytosis

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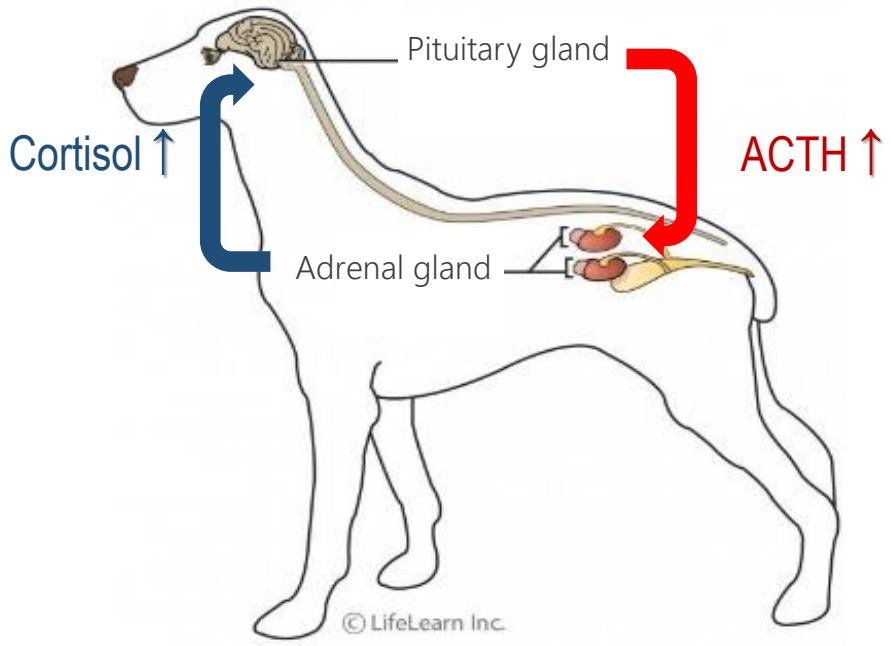
(1) Becker, Daniel E. "Basic and clinical pharmacology of glucocorticosteroids." Anesthesia progress 60.1 (2013): 25-32.

01 What is Cortisol?

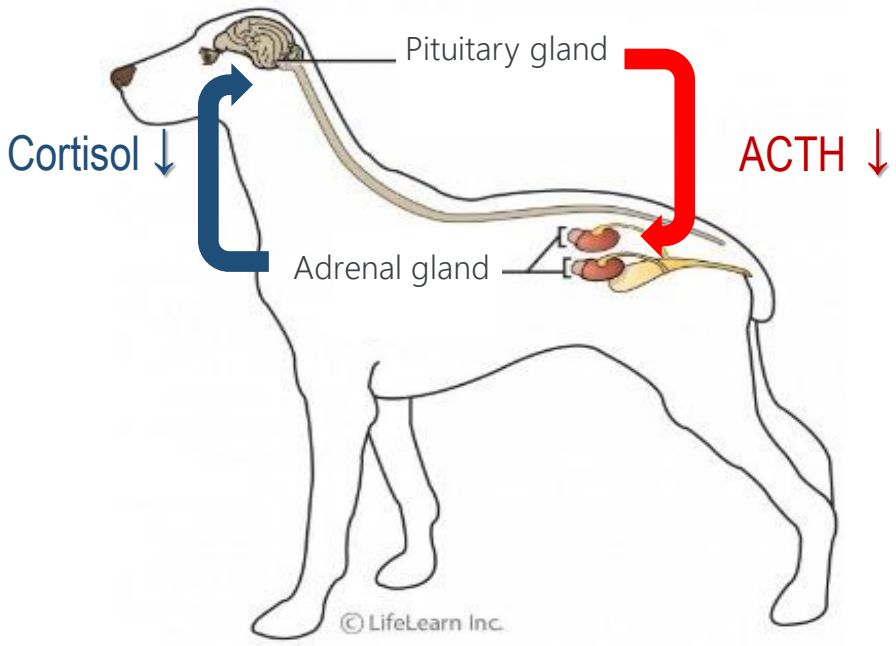
Regulation of cortisol

- Negative feedback

Cortisol concentration: Low



Cortisol concentration: High



01 What is Cortisol?

Cortisol disease

Cortisol concentration: Low

Addison's disease
(Hypoadrenocorticism)

Cortisol concentration: High

Cushing's disease
(Hyperadrenocorticism)

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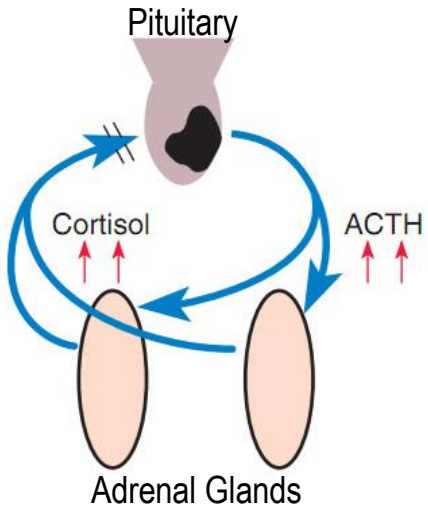
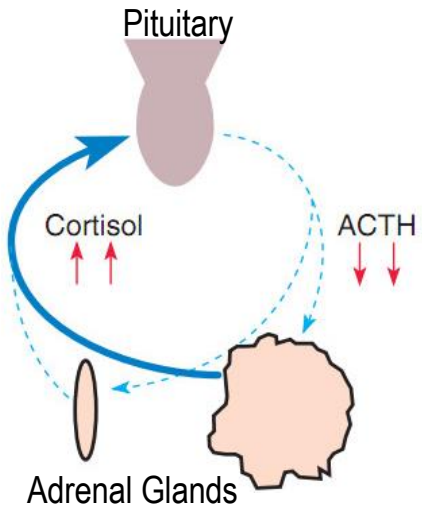
02 Cushing's disease

Cushing's disease

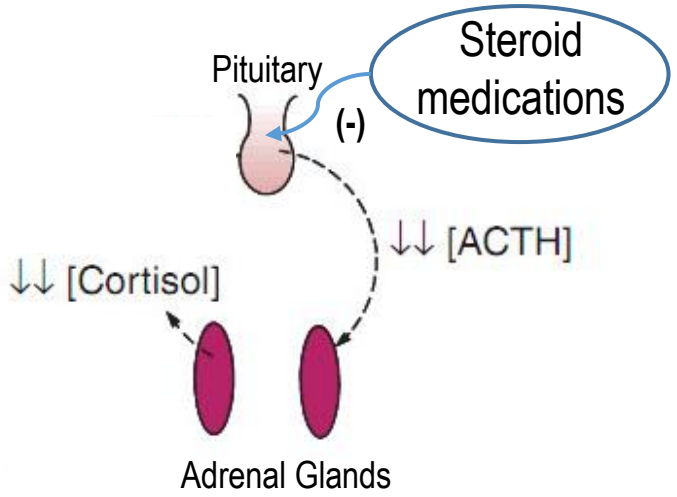
- The **most common endocrinopathy** of older dogs
- Caused by excessive secretion or exogenous administration of glucocorticoids



① **Pituitary dependent**
(PDH, 80~85%)



② **Adrenocortical dependent**
(adrenal tumor AT, 15~20%)



③ Iatrogenic Cushing's
Excessive administration of glucocorticoid

Nelson, R. W., & Couto, C. G. (2014). Small Animal Internal Medicine-E-Book. Elsevier Health Sciences.

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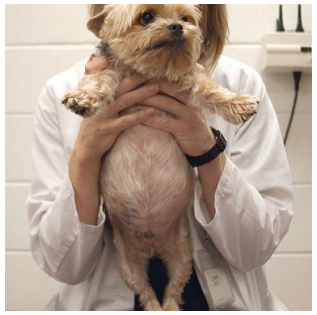
02 Cushing's disease

Clinical signs

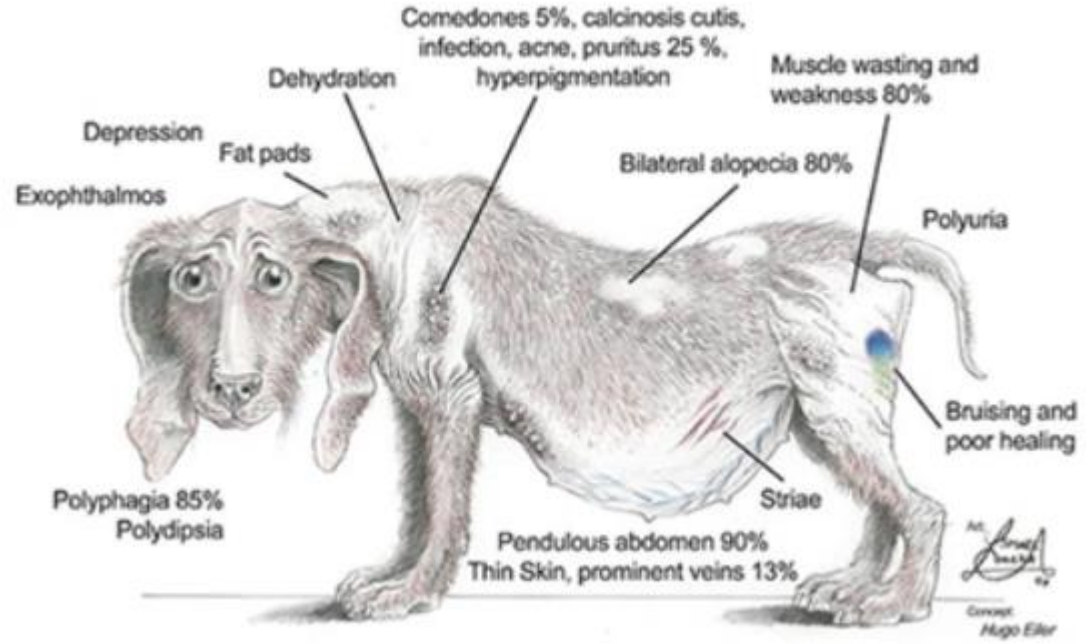
- **General**

Check Five 'P's

- ✓ Polyuria/Polydipsia (> 95%)
- ✓ Polyphagia
- ✓ Abdominal enlargement ("Pot-belly")
- ✓ Panting
- ✓ Muscle wasting & weakness



- **Dermatologic**
  - ✓ Calcinosis cutis
  - ✓ Thin skin



By Audrey Cook, Today's Veterinary Practice

02 Cushing's disease

Laboratory findings

- ✓ CBC
  - Normal or Stress leukogram
  - +/- Increased platelet count
- ✓ Serum chemistry
  - Increased **ALKP activity** (~85%)
  - Increased cholesterol, triglyceride
  - +/- Hyperglycemia (mild)
- ✓ Urinalysis
  - **Low USG** (urine specific gravity) < 1.020
  - Bacteriuria, Proteinuria (UPC)

Parameters	Stress	Inflammation
WBC Count	High	High
Neutrophils	High	High
Lymphocytes	Low	Normal
Eosinophils	Low	Normal
Monocytes	Slightly high	Slightly high

▲ Stress leukogram

With a strong clinical index of suspicion,
- Polyuria / Polydipsia / Polyphagia
- Dog without other diseases

Diagnosis of Cushing's should be made!

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02 Cushing's disease

Diagnostic Approach

- **Screening** : Diagnosis of Cushing's

  ① **UCCR**

  ② **LDDST**

  ③ ACTH Test

- **Differentiating** : Differentiation PDH and AT

  ① **Ultrasonography**

  ② Endogenous ACTH

✓ **Not recommended**

- ACTH: Useful for diagnosis of Addison disease, Iatrogenic Cushing's, treatment monitoring(Cushing's)
- Change of trend: ACTH → LDDST
- HDDST: Cannot differentiate PDH and AT in many cases

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02 Cushing's disease

Screening

① **UCCR testing** (Urine Cortisol to Creatinine ratio)

- ✓ Have the owner collect morning urine **at home** (minimizing stress or fear)
  → Centrifuge → Send it the lab service

- ✓ **Overly sensitive (> 97%)**, but **Not very specific**
  - Negative result ☞ can be used to exclude it with accuracy
  - Positive result ☞ can be false;

- ✓ **False positive results are common** → Further testing (LDDST) should be performed

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02 Cushing's disease

Screening

② **LDDST** (Low dose dexamethasone suppression test)

- ✓ **Gold standard method, Highly sensitive (85-100%)**
- ✓ **Test procedure**
  - ① Collect a baseline cortisol sample
  - ② 0.01 mg/kg of **dexamethasone** IV (saline dilution)
  - ③ Collect blood samples at 4, 8 hours
- ✓ **Test time: 8 hours**
- ✓ **Differentiates PDH from AT** (only about 25% cases)
- ✓ **Caution:** After PDS withdrawal (2-4 weeks)

For more information, please click below link!
http://bionote.co.kr/eng/board/vcheck_reagent/board_view.asp?page=1&num=288

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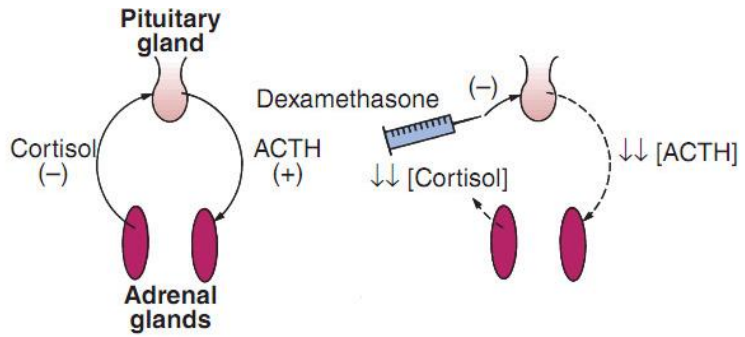
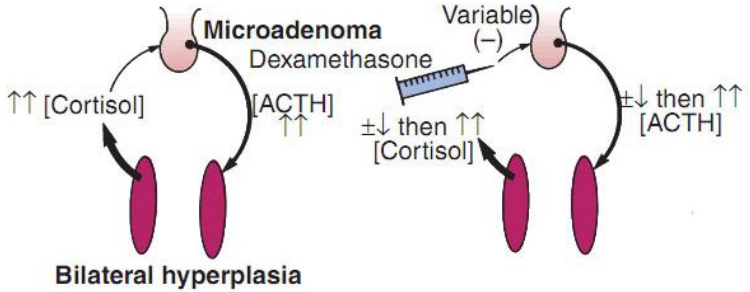
02 Cushing's disease

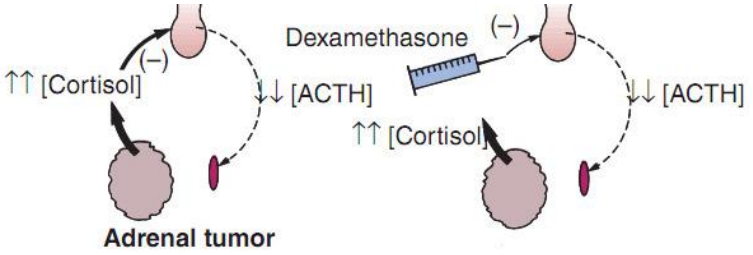
Normal

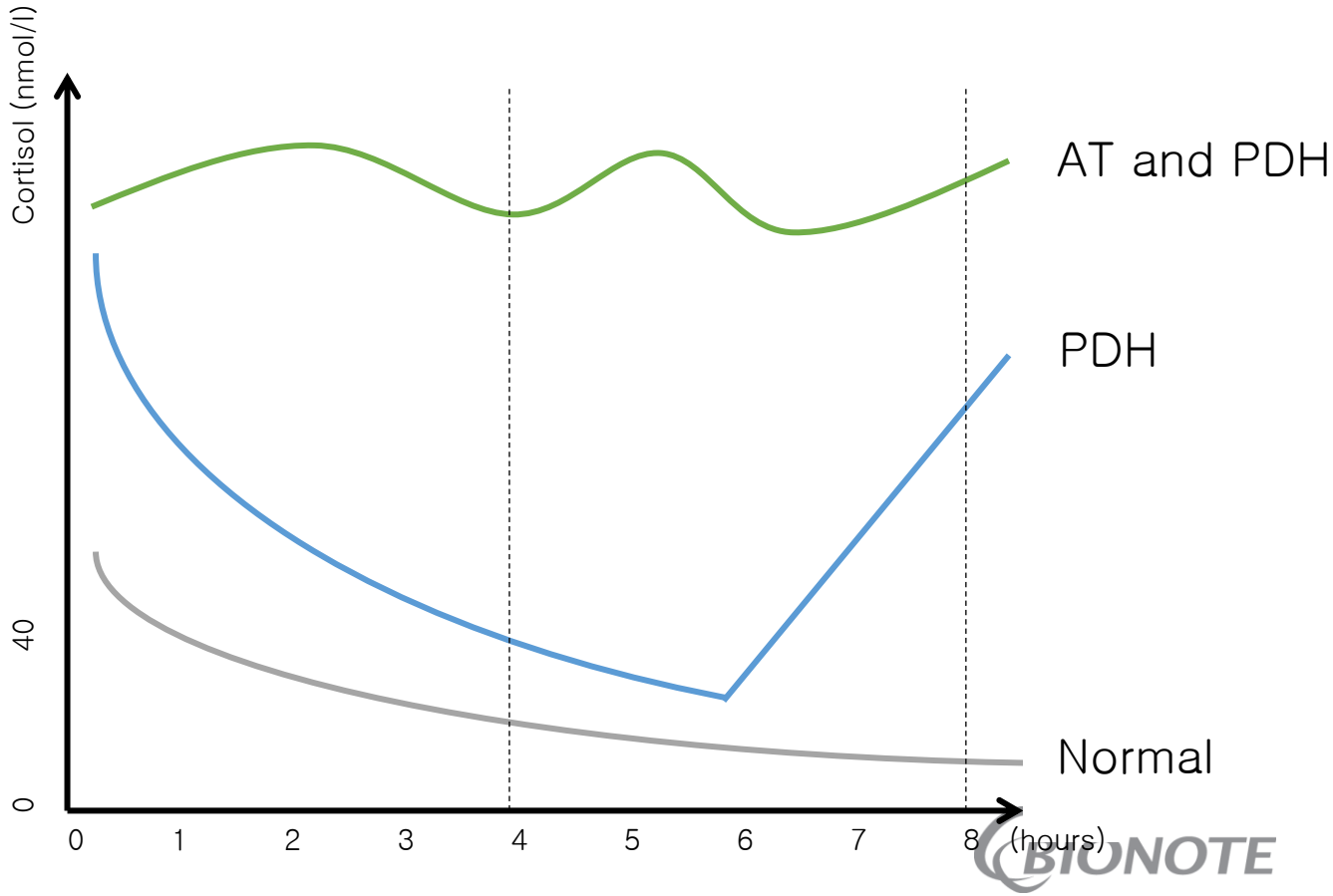


PDH



AT



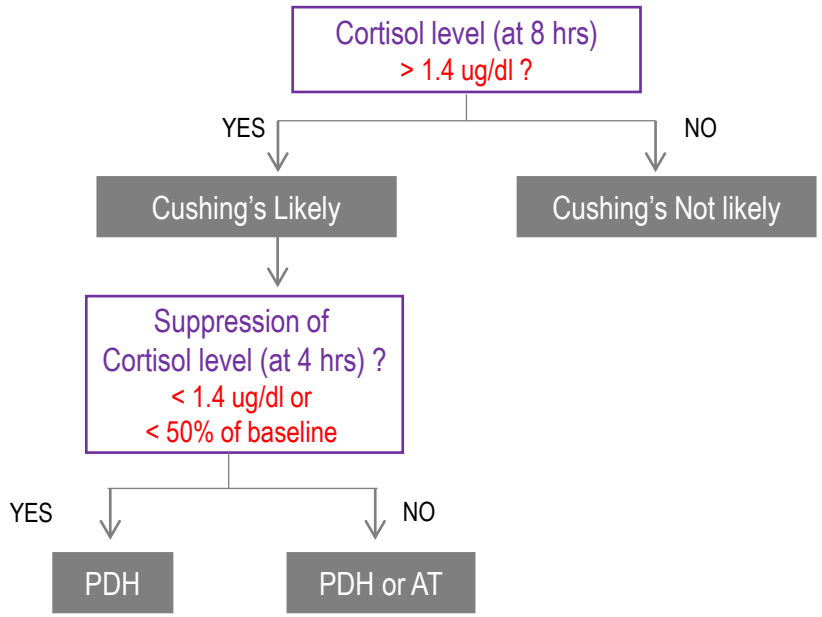


02 Cushing's disease

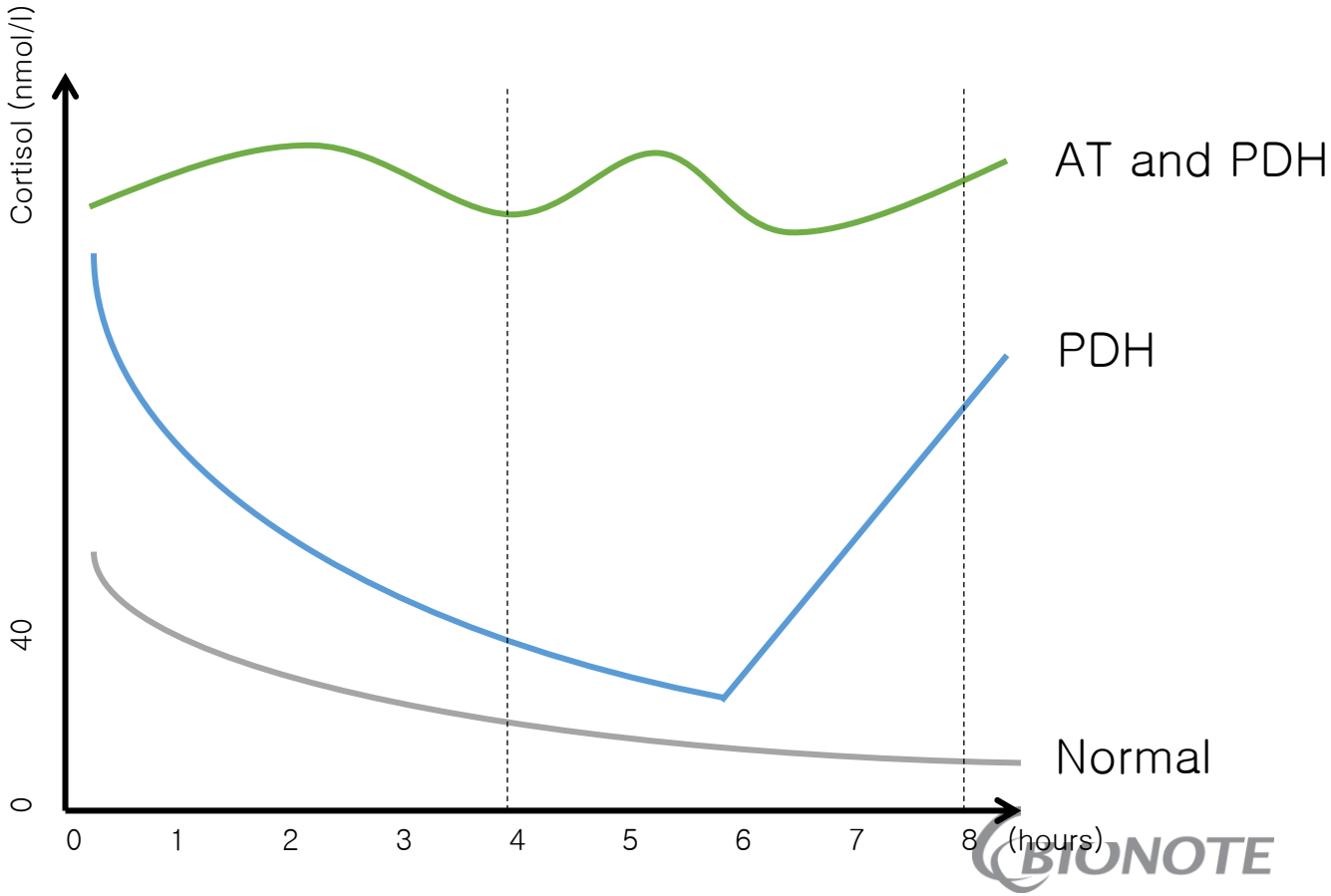
Screening

② LDDST (Low dose dexamethasone suppression test)

✓ **Result Interpretation**



*Failure to suppress at the 4-hour mark is not diagnostically useful; it is seen in many dogs with PDH and all those with AT.

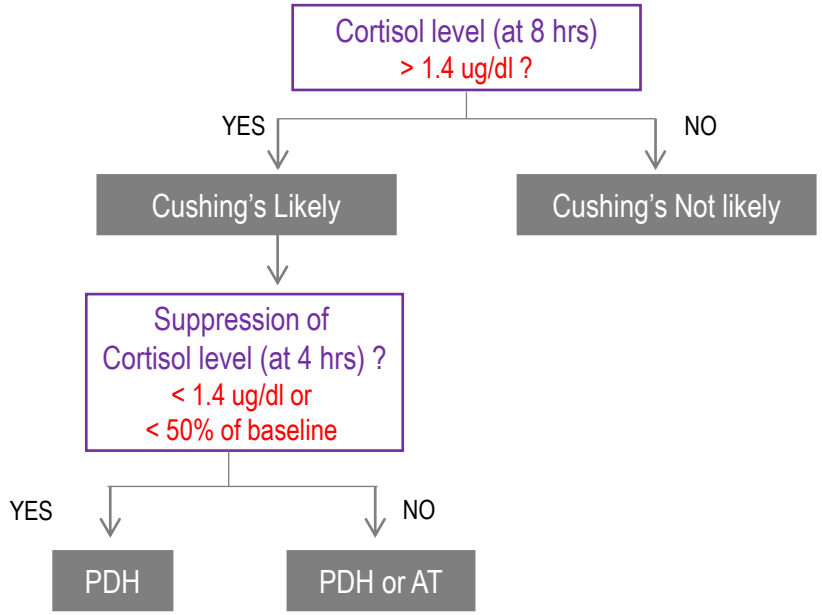


02 Cushing's disease

Screening

② LDDST (Low dose dexamethasone suppression test)

✓ **Result Interpretation**



*Failure to suppress at the 4-hour mark is not diagnostically useful; it is seen in many dogs with PDH and all those with AT.

✓ **Quiz**

- A patient with PU/PD/PP who was strongly suspected with Cusing's
- **LDDST testing performed**:

  0H(Pre injection) **8.6**

  4H cortisol **3.1**

  8H cortisol **3.6**

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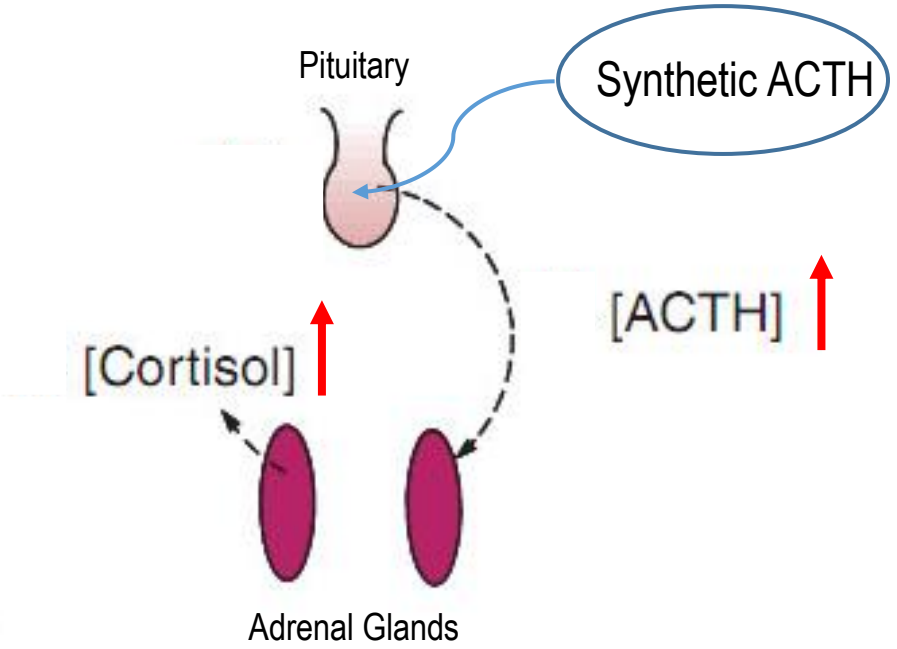
02 Cushing's disease

Screening

③ ACTH Test

- ✓ **Screening test for Cushing's disease**
- ✓ **Test procedure**
  - ① Collect a baseline cortisol sample
  - ② 0.25 mg synthetic ACTH
  - ③ Collect blood samples at 1 hour after
- ✓ **Test time: 1 hour**
- ✓ **Cannot differentiate PDH and AT**
- ✓ **Accuracy: ACTH < LDDST**
- ✓ **<u>Caution</u>:** After PDS withdrawal (2-4 weeks)



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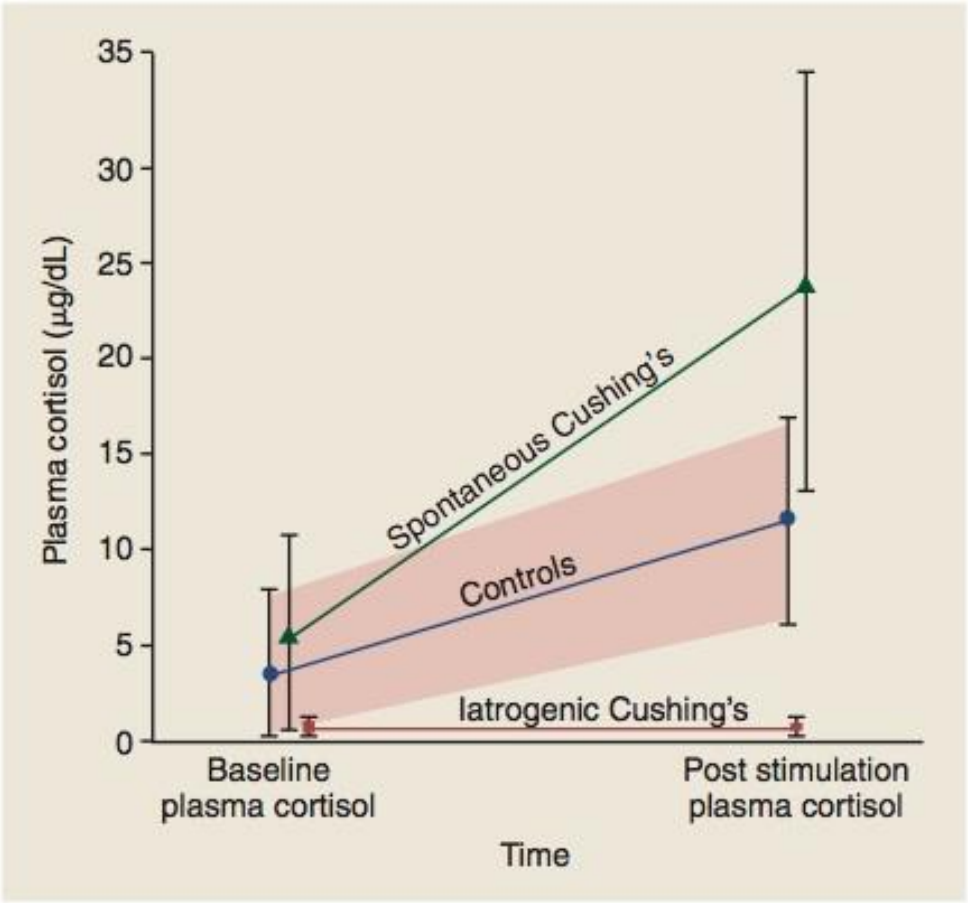
02 Cushing's disease

Screening

③ ACTH Test

Pre-ACTH	Interpretation	Post-ACTH	Interpretation
< 2 μg/dL	If Pre- and post-ACTH results are < 2 μg/dL, results are consistent with hypoadrenocorticism	< 2 μg/dL	If Pre- and post-ACTH results are < 2 μg/dL, consistent with hypoadrenocorticism
2-6 μg/dL	Normal	2-6 μg/dL	Equivocal
		6-18 μg/dL	Normal
		18-24 μg/dL	Equivocal
		> 24 μg/dL	Consistent with hyperadrenocorticism

LDDST



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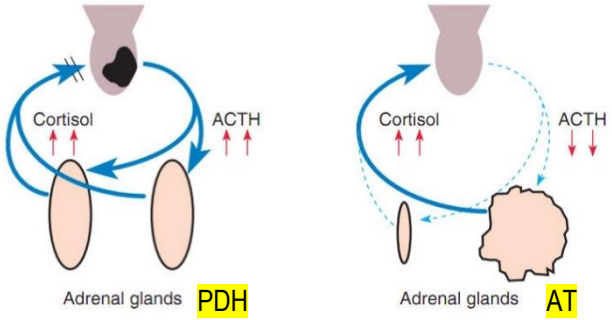
02 Cushing's disease

Differentiation Testing

① Abdominal ultrasound



- ✓ **Useful testing for differentiating PDF from AT**
- ✓ **Check list:** adrenal size (> 6 mm), symmetry, invasion of adjacent structures, etc.

❖ **PDH**

- Bilateral adrenal hypertrophy

❖ **AT**

- Unilateral adrenal hypertrophy
- Atrophy of contra-lateral gland
- Nodular change

▲ Ultrasound image of adrenal adenoma

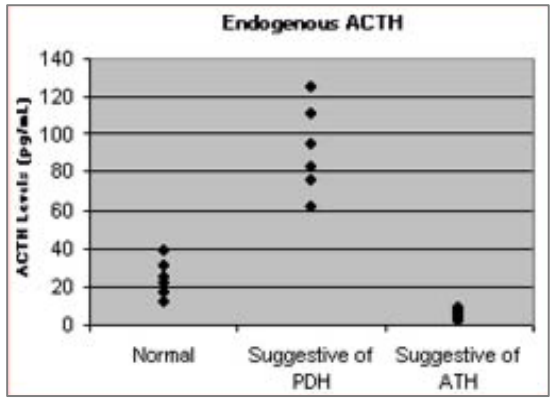
By David Bruyette, Today's Veterinary Practice

02 Cushing's disease

Differentiation Testing

② Endogenous ACTH

- ✓ **Used to distinguish between PDH and AT**
- ✓ **Highly sensitive & specific**
  - Normal, Increased level ☞ PDH
  - Low level ☞ AT
- ✓ eACTH is a fragile hormone, so careful sample handling is essential



eACTH	Interpretation
10 – 80 pg/mL	Normal
> 45 pg/mL	PDH
< 20 pg/mL	AT

02 Cushing's disease

Monitoring

- **ACTH stimulation test**

1. **Starting dose:** 2.2 mg/kg SID, with food

2. **Action after 10-14 days at this dose**
   - **ACTH ST:** 4-6 hour post-dosing
   - Serum biochemistry + electrolytes + USG

Post-ACTH serum cortisol	Action
< 1.45 ug/dl (< 40 nmol/L)	Stop treatment. Re-start at a decreased dose
1.45 - 5.4 ug/dl (< 40 - 150 nmol/L)	Continue on same dose
5.4 – 9.1 ug/dl (< 150 - 250 nmol/L)	① If clinical signs are well controlled, continue on current dose ② If clinical signs are still evident, Increase dose
> 9.1 ug/dl (> 250 nmol/L)	Increase initial dose

3. **Individual dose adjustments**
   - Conduct an ACTH ST 10-14 days after every dose alteration
   - **If clinical signs are not controlled for the full day?** Twice daily dosing may be needed.

4. **Long term monitoring**
   - Once an optimum dose has been reached, re-examine the dog at 30 days, 90 days and every 3 months thereafter

Good control is as follows:

① Favorable clinical signs
② post-ACTH serum cortisol of **1.45-9.1 μg/dL** (40-250 nmol/L)
③ USG ≥ 1.020

For more information, please click below link!
https://www.dechra-us.com/Files/Files/SupportMaterialDownloads/US/Vetoryl_Package_Insert.pdf

By Vetoryl (Trilostane) Insert

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02 Cushing's disease

Case Study

✓ **Signalment**
- 12-year-old, female, Miniature Schnauzer

✓ **Chief Complaint**
- **Polyphagia, weight gain, PU/PD, panting at rest**

✓ **Physical examination**
- Abdominal distention, muscle weakness

✓ **Hematologic**
- **High ALP** (>3500 U/l)
- Hypercholesterolemia (435 mg/dl)
- Hypertriglyceridemia (>500 mg/dl)
- Hyperglycemia (175 mg/dl)

✓ **Urinalysis**
- **USG 1.011 (Reference: 1.015-1.045)**

✓ **Hormone testing (LDDST)**

LDDST	Reference	Result
Basal cortisol	2.0 – 6.0	8.9 ug/dl
4H cortisol		2.9 ug/dl
8H cortisol		4.2 ug/dl

✓ **Treatment**
- Start with trilostane 2 mg/kg BID

✓ **Monitoring (ACTH ST)**

	ACTH ST	Reference	Result
2 weeks later	Pre-cortisol	2.0 – 6.0	4.9
	Post-cortisol	1.45 - 5.4	2.6

☞ dosage reduced (trilostane 1 mg/kg BID)

	ACTH ST	Reference	Result
11 days later	Pre-cortisol	2.0 – 6.0	4.1
	Post-cortisol	1.45 - 5.4	5.5

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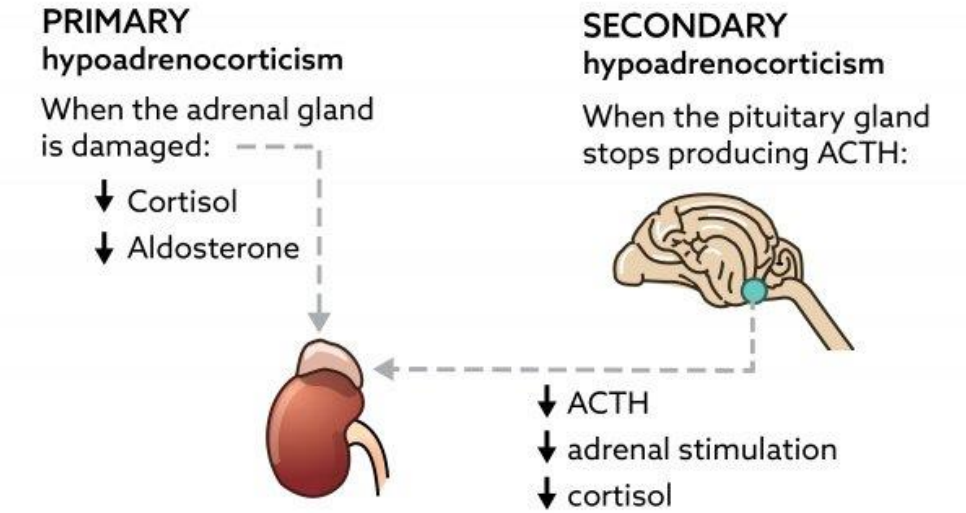
03 Addison's disease

- Occurs when the adrenal glands fail to produce the hormones

Classification

- **Primary (95%)**
  - **Cause**: Immune-mediated, drugs (e.g. mitotane)
  - Bilateral destruction of the adrenal cortex
    - ☞ Reduced secretion of both cortisol and aldosterone

- **Secondary (5%)**
  - **Cause**: Pituitary disease
  - Reduction of ACTH hormone secretion in pituitary gland
    - ☞ Reduced secretion of cortisol only



Clinical signs

- **Waxing and waning**
- **Nonspecific**
  - Anorexia, lethargy, weakness, weight loss
  - Vomiting, diarrhea

By Krista Williams, BSc, DVM, CCRP / Vcahospitals

03 Addison's disease

Clinicopathologic

Common clinicopathological abnormalities in 267 dogs with hypoadrenocorticism*

Finding	*Number of dogs*
Hyperkalemia	251 (94%)
Hyponatremia	219 (82%)
Sodium:potassium ratio < 27	252 (94%)
Hypochloremia	123 (46%)
Hypercalcemia	77 (29%)
Azotemia	231 (87%)
High serum alanine aminotransferase (ALT)	85 (32%)
Hyperbilirubinemia	53 (20%)
Hypoglycemia	52 (19%)
Anemia	66 (25%)
Eosinophilia	56 (21%)
Lymphocytosis	24 (9%)
Urine specific gravity < 1.030 (in face of azotemia)	112 (42%)

*Compiled from data in references 4 and 7.

WALTHAM FOCUS VOL8 NO1 1998

Cortisol testing

- **Basal cortisol** (Screening)

Basal Cortisol	Sensitivity	Specificity
≤ 0.2 ug/dL	81.8%	99.1%
≤ 0.8 ug/dL	96.9%	95.7%
≤ 1.0 ug/dL	98.2%	91.5%
≤ 1.4 ug/dL	99.4%	82.6%
≤ 2.0 ug/dL	99.4%	67.0%

☞ Recommended

☞ False-positive results…

A.J. Gold D.K. Langlois. Journal of Veterinary Internal Medicine 2016

- **ACTH stimulation test** (Reference)
  - Gold standard method for diagnosis
  - **If pre- and post-ACTH results are < 2 ug/dL,**
    - ☞ Consistent with hypoadrenocorticism

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04 Product introduction

- Specifications & Performance
- Test Procedure
- Reference Range & Interpretation
- Quiz

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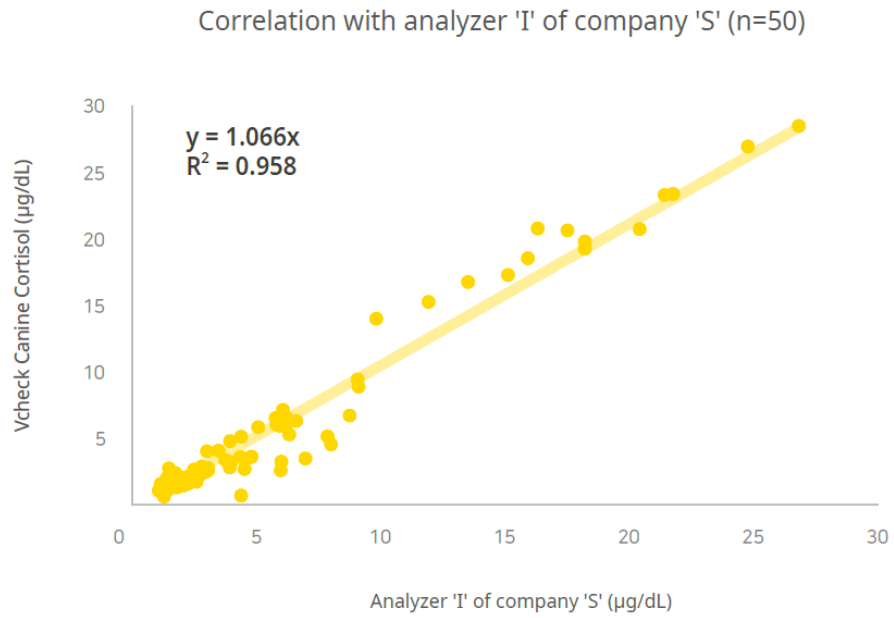
04 Product introduction

- Specifications

- ✓ **Species** : Dog
- ✓ **Sample** : Serum 50 µl
- ✓ **Testing Time** : 20 minutes
- ✓ **Storage**: 2-8 ℃
- ✓ **Measurement** : Quantitative (1 ~ 30 ug/dl)

- Performance



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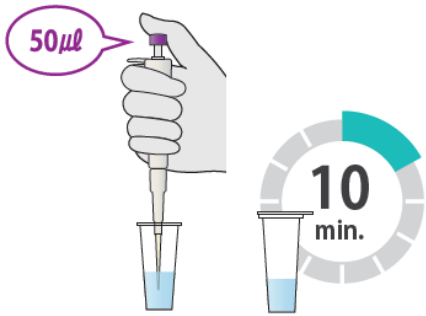
04 Product introduction

- Test procedure

Dilute sample

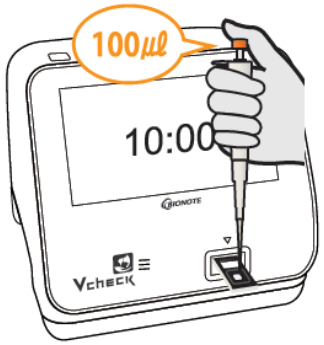
Using a 50 ㎕ pipette, draw 50 ㎕ of serum and add to the assay diluent tube.

Mix & Incubate



Mix the sample and diluent. Incubate the sample mixture at room temperature for 10 minutes.

Measure



Add 100 ㎕ of incubated sample to the sample well of test device using a 100 ㎕ pipette and press [START].

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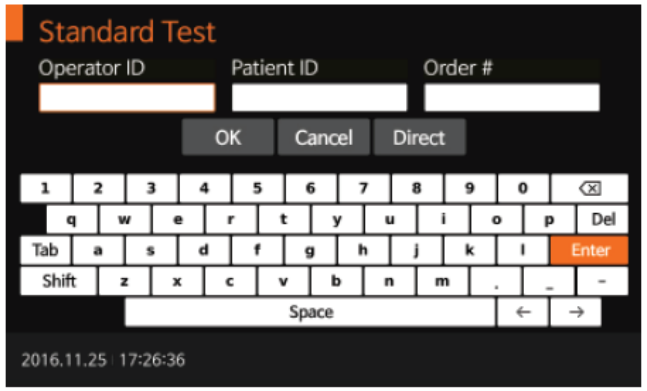
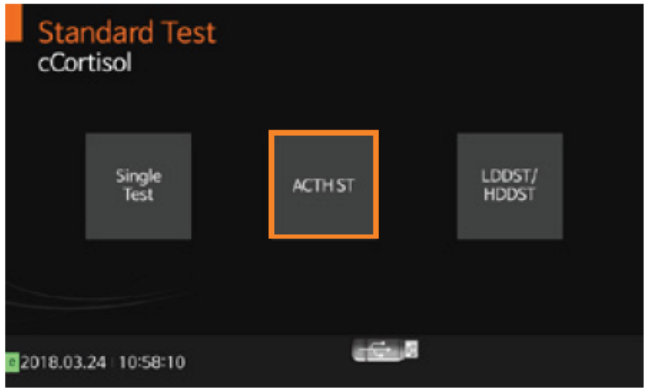
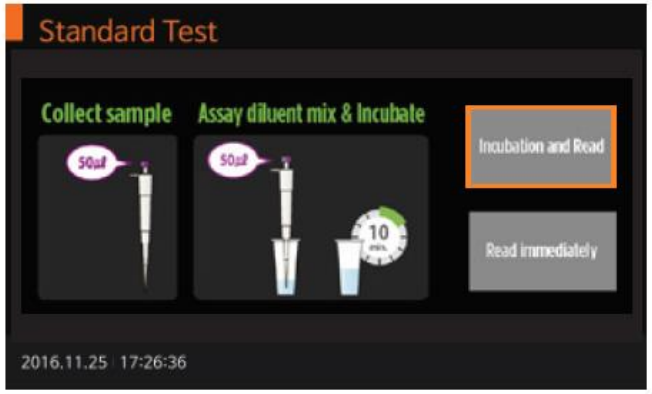
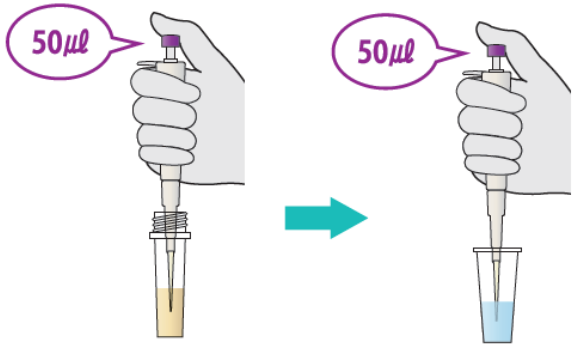
04 Product introduction

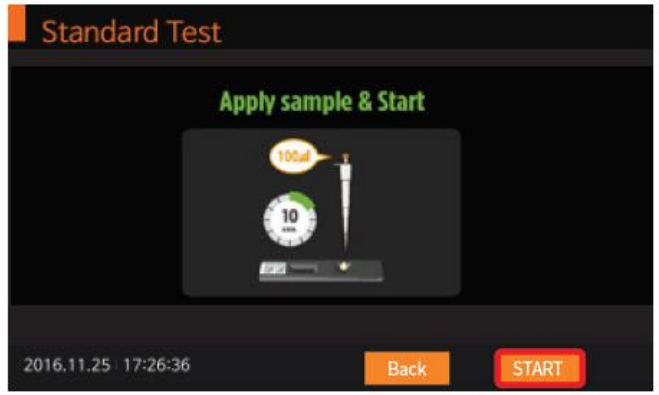
- Test procedure – ACTH





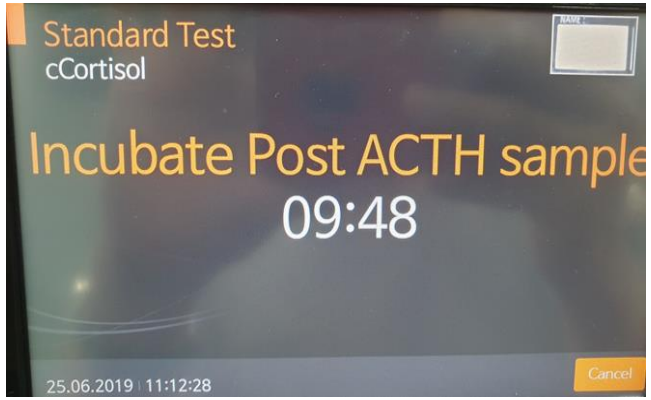


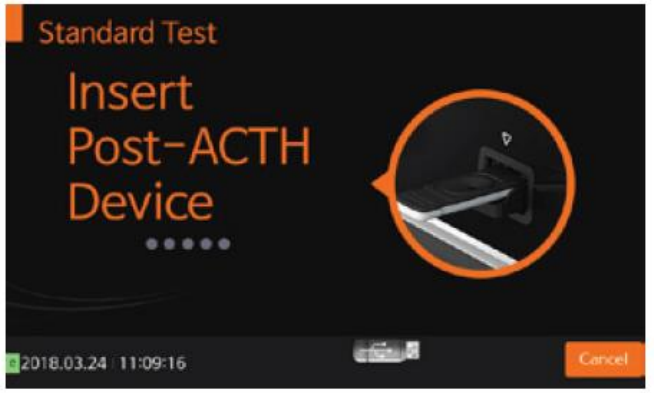


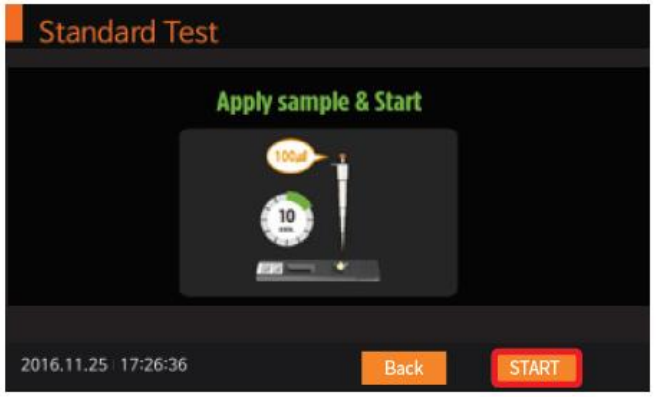


04 Product introduction

- Test procedure – ACTH

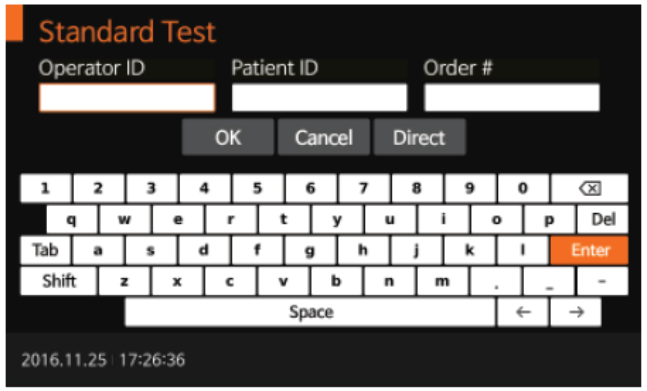


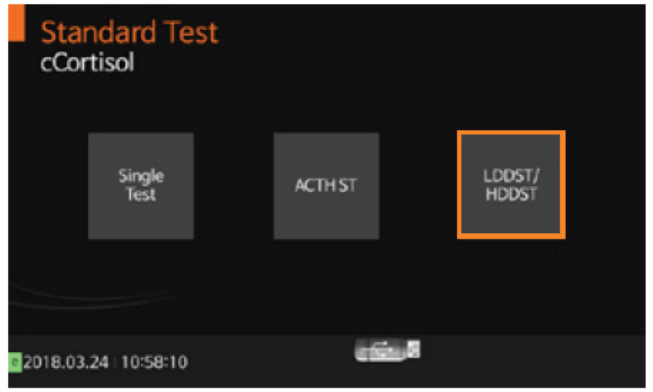


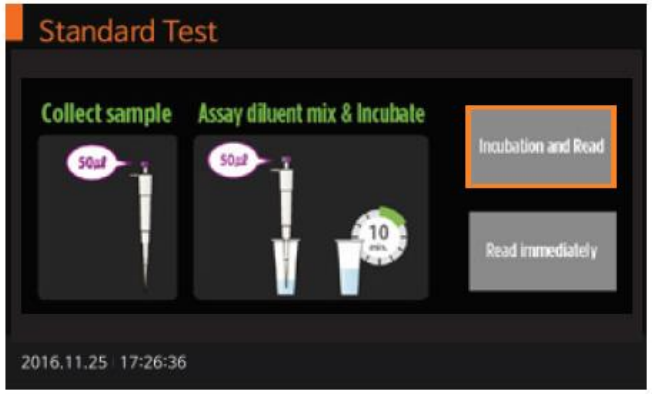


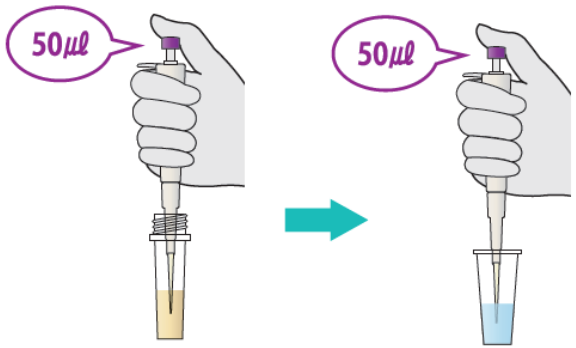
04 Product introduction

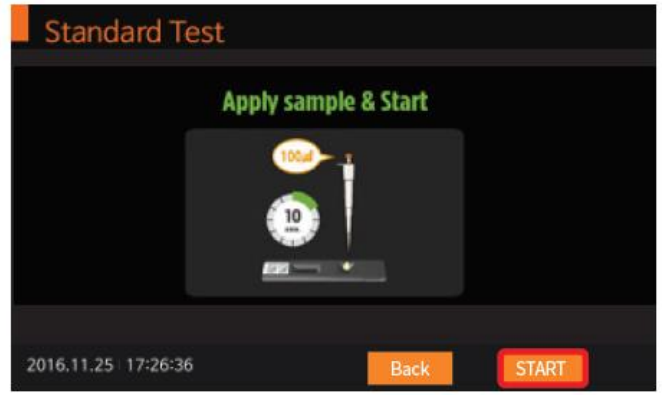
- Test procedure – LDDST





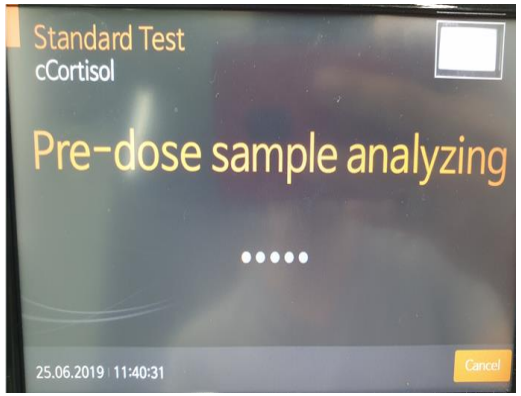


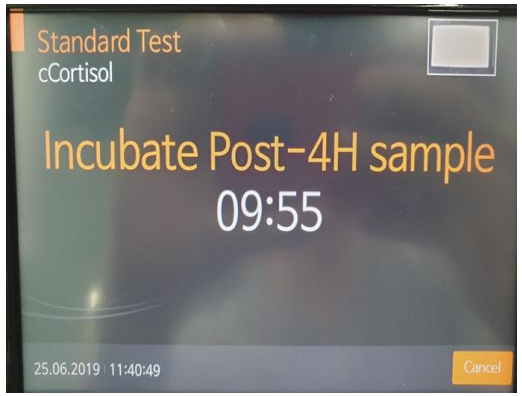


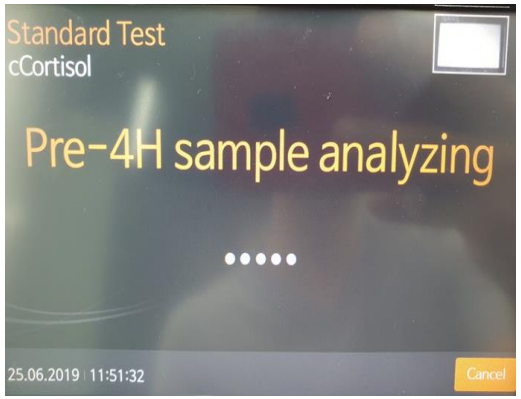


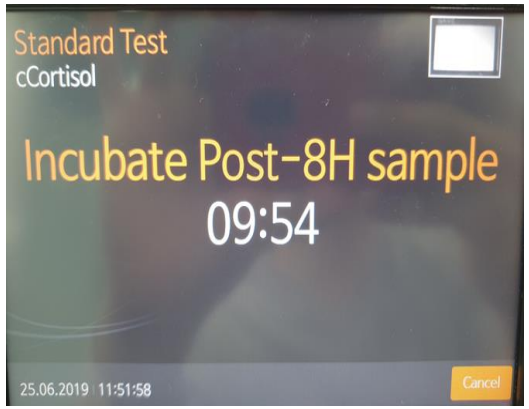
04 Product introduction

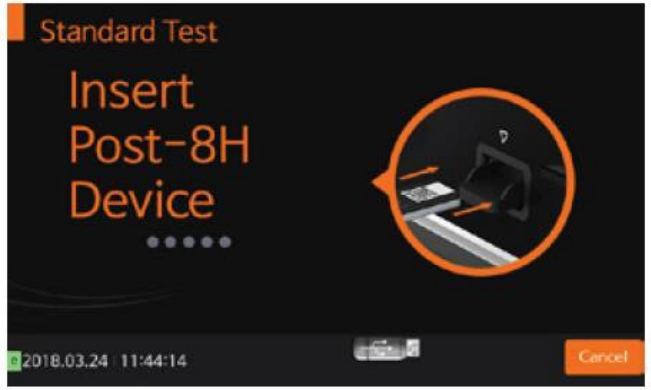
- Test procedure – LDDST











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04 Product introduction

- Test procedure – LDDST

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04 Product introduction

- Reference range

[ACTH stimulation Test] *For monitoring*

Pre-ACTH	Interpretation	Post-ACTH	Interpretation
< 2 μg/dL	If Pre- and post-ACTH results are < 2 μg/dL, results are consistent with hypoadrenocorticism	< 2 μg/dL	If Pre- and post-ACTH results are < 2 μg/dL, consistent with hypoadrenocorticism
2-6 μg/dL	Normal	2-6 μg/dL	Equivocal
		6-18 μg/dL	Normal
		18-24 μg/dL	Equivocal
		> 24 μg/dL	Consistent with hyperadrenocorticism

[Low-Dose Dexamethasone Suppression Test] *For diagnosis*

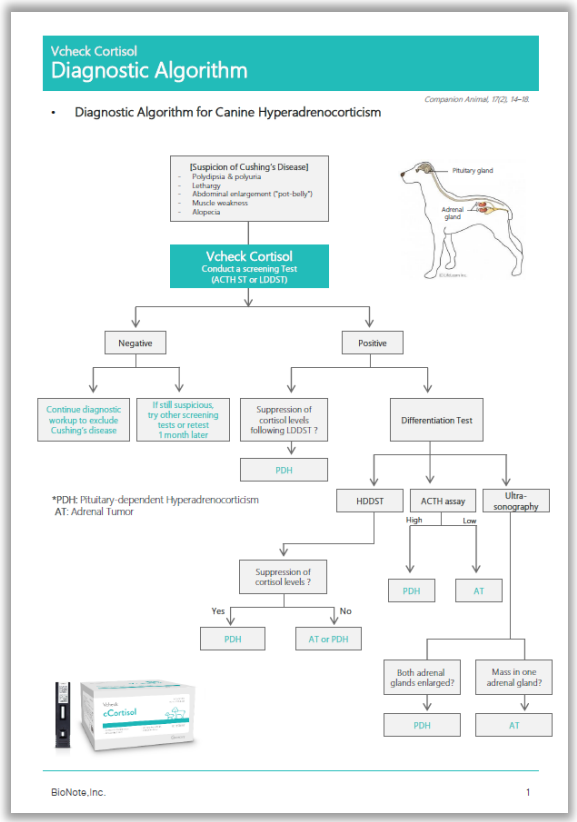
4-hour cortisol level	8-hour cortisol level	Interpretation
-	< 1 μg/dL	Normal
1-1.4 μg/dL	1-1.4 μg/dL	Equivocal
> 1.4 μg/dL and > 50% of baseline	> 1.4 μg/dL and > 50% of baseline	Consistent with Hyperadrenocorticism
< 1.4 μg/dL or < 50% of baseline	> 1.4 μg/dL and > 50% of baseline	Consistent with PDH
> 1.4 μg/dL or > 50% of baseline	> 1.4 μg/dL and < 50% of baseline	Consistent with PDH
< 1.4 μg/dL or < 50% of baseline	> 1.4 μg/dL and < 50% of baseline	Consistent with PDH

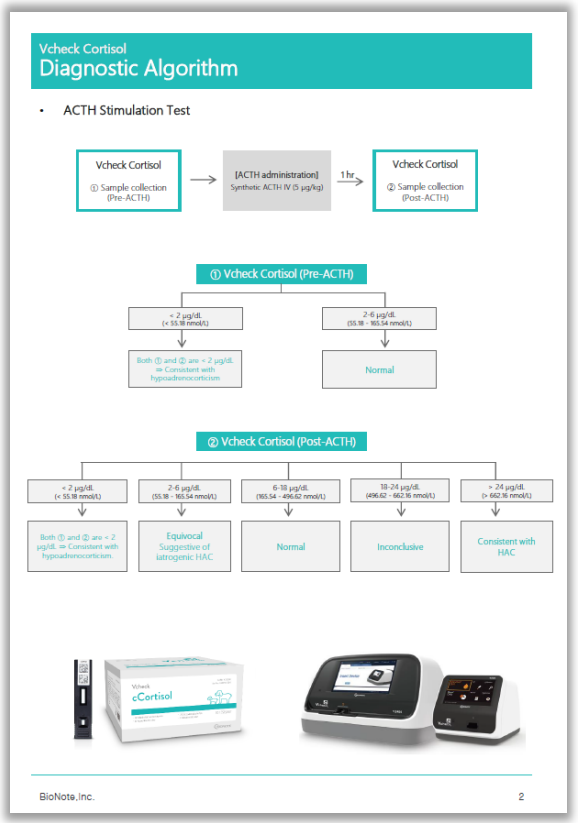
- Interpretation

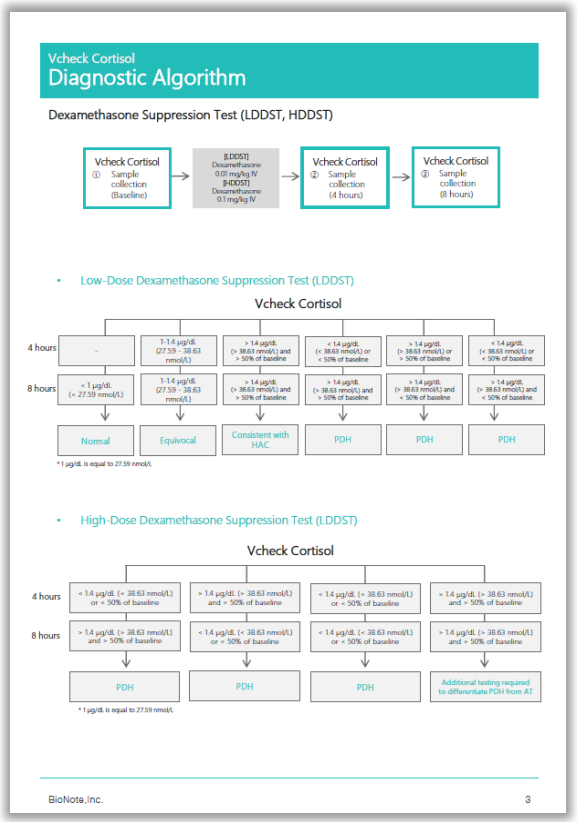
04 Product introduction

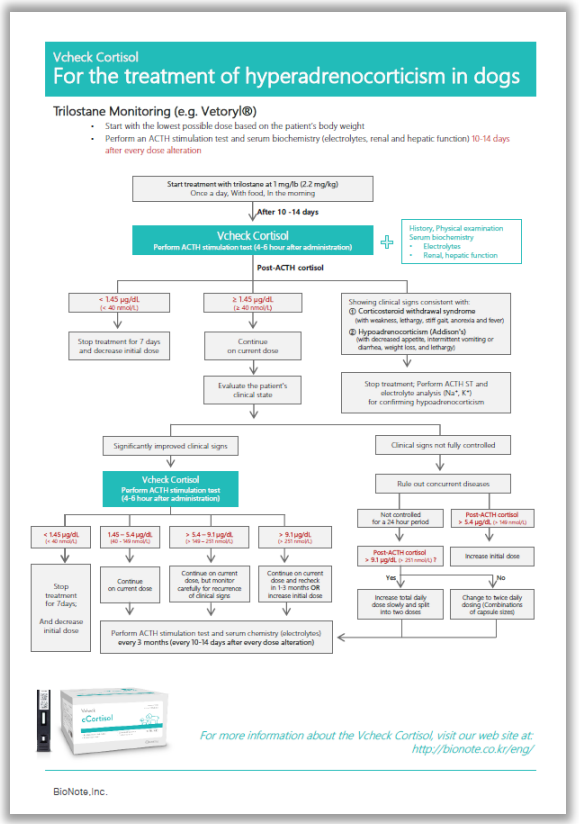
- **Clinical guidelines**
  From diagnosis to monitoring

By BioNote









04 Product introduction

Quiz

1. Is the urine sample available in Vcheck cCortisol kit?

   No. Only serum sample is available in this kit. The UCCR test result is calculated based on the ratio of creatinine and cortisol. For this test, you can refer the sample to laboratories.

2. A dog is suffering from other disease. Can I do the LDDST testing in this dog?

   No. It is recommended that the diagnosis of cushing's disease should be done after controlling other diseases. Other diseases can cause the false-positive results considering that the cortisol level can be increased in these situations.

3. In a dog with clear clinical signs, ACTH stimulation test result indicates that the dog has equivocal cushing's disease. Can I diagnose the cushing's?

   No. For starting the trilostane treatment, the definitive diagnosis should be done. ACTH stimulation test is not recommended for the diagnosis. For accurate diagnosis, LDDST testing should be considered.

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Thank you

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