Vcheck
Feline NT-proBNP

BIONOTE Marketing team

Mar. 2020

BIONOTE

Vcheck Feline NT-proBNP
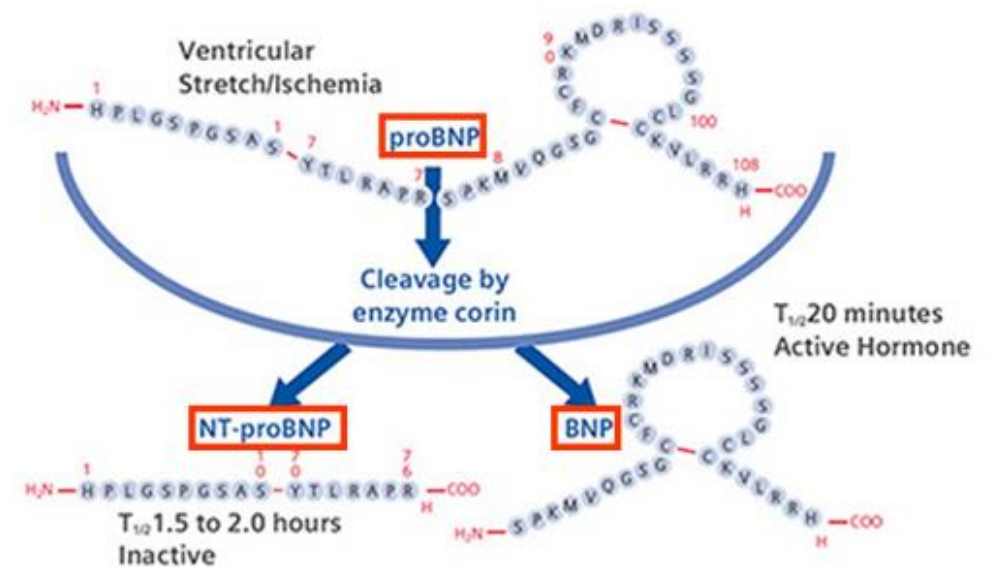
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01 NT-proBNP

- What is NT-proBNP?

- What NT-proBNP levels tell us

- How can I use the NT-proBNP test?

- <u>Algorithm</u>: NT-proBNP testing in cats

- Factors which increase NT-proBNP concentration

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What is NT-proBNP?

- pro B-type natriuretic peptide (proBNP)

  - produced in the muscle cells of the heart

  - <u>increases with excessive stretching of the cells</u>

    ⇒ correlated to the severity of the underlying heart disease

- proBNP is cleaved into <u>BNP</u> and <u>NT-proBNP</u>

  (※ NT-proBNP: N-terminal pro-B type natriuretic peptide)

- NT-proBNP is stable and has a long half-life, making it a more desirable biomarker.

  ⇒ used to assess the magnitude of cardiac muscle stretching

  ⇒ proportionate to the severity of cardiac disease

NT-proBNP in Cats

What NT-proBNP levels tell us

- **To screen for occult heart disease**
  - ✓ Before anesthesia
  - ✓ In apparently healthy cats with heart murmurs
  - ✓ At risk breeds - Maine Coon, Ragdoll, Birman, Persian, etc.

- **To determine Cardiac or Respiratory disease**
  - ✓ In cats with respiratory signs such as dyspnea, tachypnea, cough
  - ✓ To differentiate cardiac and respiratory causes of dyspnea

- **To determine the severity of heart disease**
  - ✓ For monitoring stabilization of CHF during hospitalization
  - ✓ For predicting survival in cats with CHF
    * CHF: Congestive Heart Failure

▲ Regular health check in a healthy cat
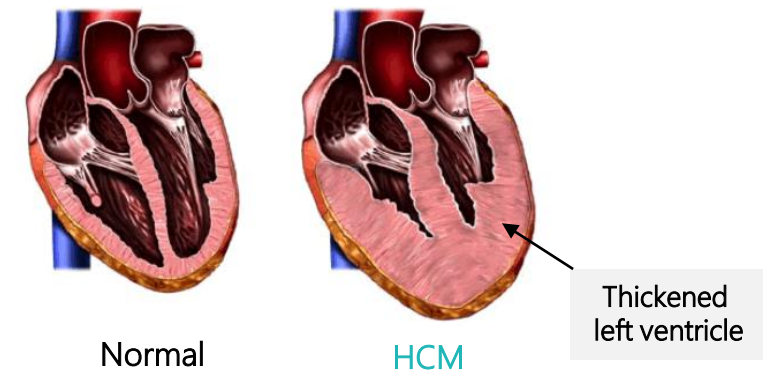
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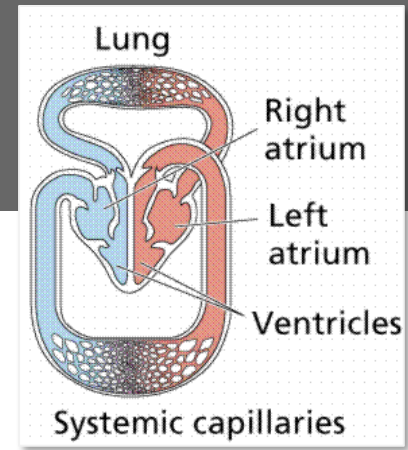
Hypertrophic Cardiomyopathy (HCM)

- Most prevalent feline cardiac disorder
- **Affected Age**: 2, 3 year-old (3 months -10 years)
- **Process:** Thick left ventricular muscle
  → Decreased volume size in left ventricle
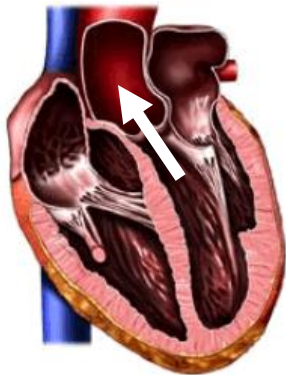
⇒ Congestive Heart Failure (CHF), Thromboembolism

Thickened left ventricle

Normal   HCM

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NT-proBNP in Cats
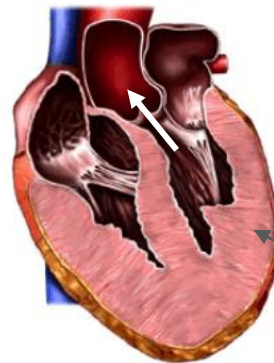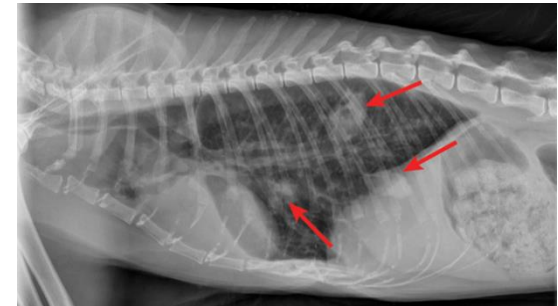
What NT-proBNP levels tell us

| Normal | → Thickening of the heart muscle | Hypertrophic Cardiomyopathy (HCM) | → Back up into the veins of the lungs | Congestive Heart Failure (CHF) |

Thickened left ventricle

- Thickened left ventricle
  ⇒ Less room available for the normal amount of blood
- Eventually leads to left atrial enlargement

- Build-up of fluid in the lungs (pulmonary edema)
- or around the lungs (pleural effusion)

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NT-proBNP in Cats

What NT-proBNP levels tell us

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▲ Regular health check in a healthy cat

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NT-proBNP in Cats

How can I use the NT-proBNP test?

01 Comprehensive Evaluation (In Cats without clinical signs)

02 In Cats with Respiratory signs

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NT-proBNP in Cats

How can I use the NT-proBNP test?

01 Comprehensive Evaluation (In Cats without clinical signs)

▲ Maine Coon (high risk for HCM)

- ✓ **Regular health check** (prior to anesthesia)

- ✓ **In cats at increased risk of having occult cardiomyopathy**
  e.g. heart murmur, gallop heart sound, arrhythmia

- ✓ **In high-risk cat breeds,**
  e.g. Domestic Short- and Longhaired cats, Maine Coon, Ragdoll, Birman, Persian, American Short Hair,
  Himalayan, Siamese, Sphinx, Burmese, etc.

- • Assesses the **likelihood of underlying heart disease in asymptomatic cats**

- • Guides the next diagnostic and therapeutic steps. ⇒ Echocardiogram should be performed for the definite diagnosis.

- • Shows 91.2% specific and 85.8% sensitive for detection of underlying heart disease
  (based on findings from echocardiographic examination, *J Vet Intern Med 2011*, in press.)

+) Although NT-proBNP levels is less than 100 pmol/L, high-risk cats should be re-evaluated annually.

▲ A cat having difficulty in breathing

NT-proBNP in Cats

How can I use the NT-proBNP test?

02 In Cats with Respiratory signs

✓ **The presence of respiratory signs (dyspnea, tachypnea, cough)**

- <u>Cardiac disease</u>: underlying cardiomyopathy and congestive heart failure (CHF)

- <u>Primary respiratory disease</u>: bronchitis/asthma, pneumonia, neoplasia, pleural space disease

- The useful option to rapidly assess **the likelihood that heart disease is present in a cat with respiratory signs**.

- In cats with respiratory signs such as dyspnea, tachypnea and cough, **if the NT-proBNP is >270 pmol/L, CHF is the most likely cause of the clinical signs**. (Concurrent respiratory disease cannot be still ruled out.)

- Cutoff value of 265 pmol/L: Sensitivity of 90.2% and Specificity of 87.9%
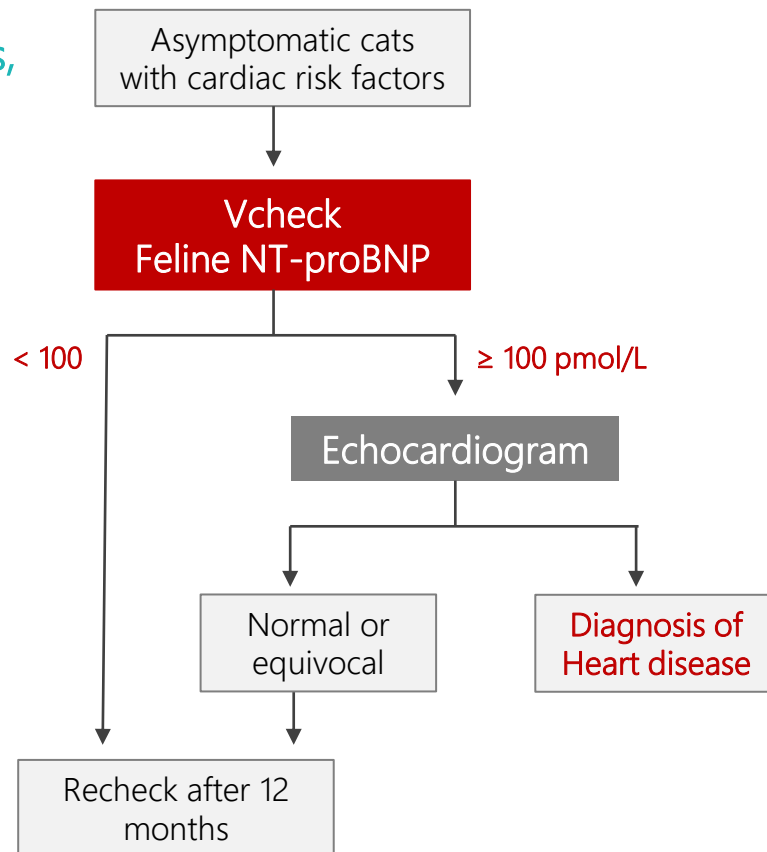
01 NT-proBNP

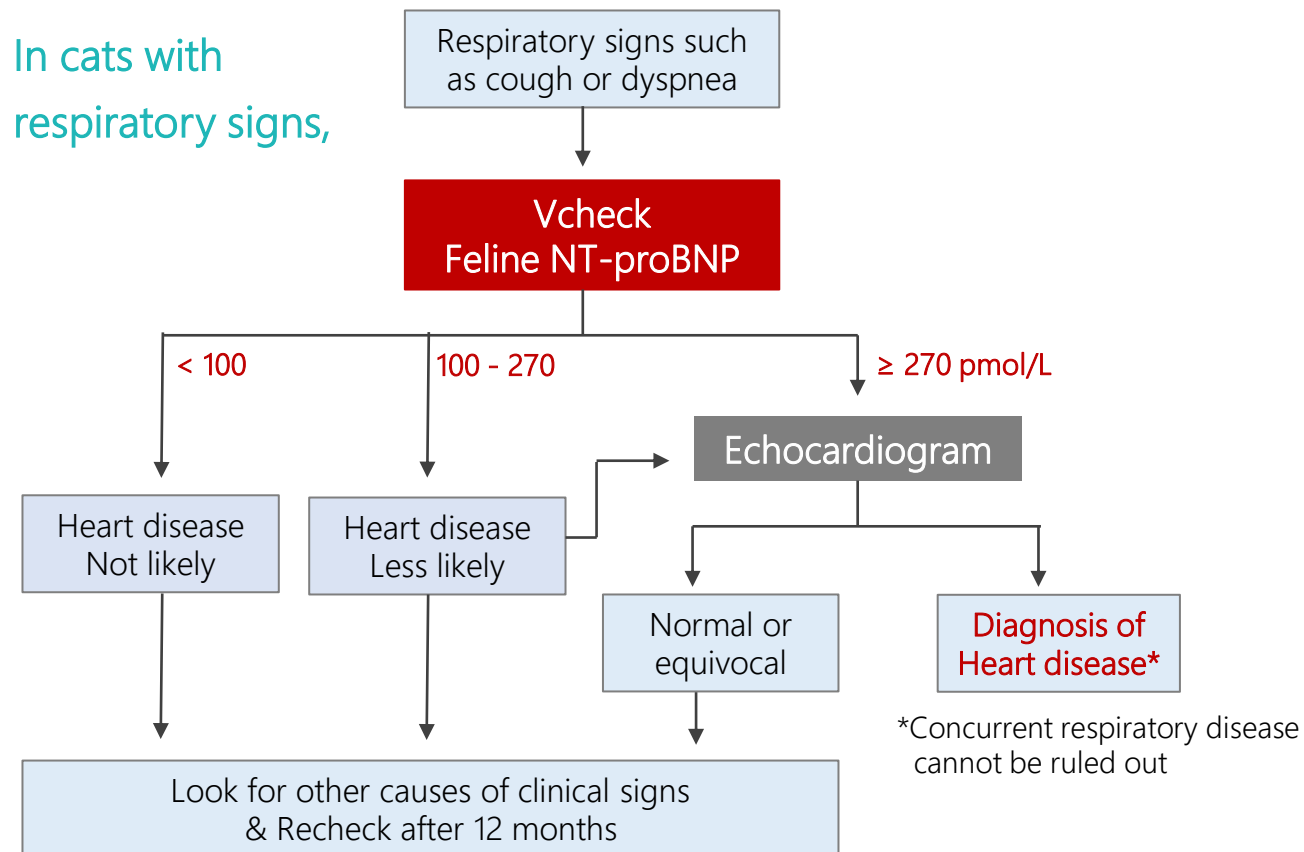## NT-proBNP in Cats

Algorithm: NT-proBNP testing in cats

Cardiac risk factors in cats
- Gallop rhythm
- Heart murmurs
- Arrhythmia
- Radiographic cardiomegaly

In healthy cats,

Asymptomatic cats with cardiac risk factors

Vcheck Feline NT-proBNP

< 100   ≥ 100 pmol/L

Echocardiogram

Normal or equivocal   Diagnosis of Heart disease

Recheck after 12 months

In cats with respiratory signs,

Respiratory signs such as cough or dyspnea

Vcheck Feline NT-proBNP

< 100   100 - 270   ≥ 270 pmol/L

Heart disease Not likely   Heart disease Less likely   Echocardiogram

Normal or equivocal   Diagnosis of Heart disease*

*Concurrent respiratory disease cannot be ruled out

Look for other causes of clinical signs & Recheck after 12 months

01 NT-proBNP

NT-proBNP in Cats
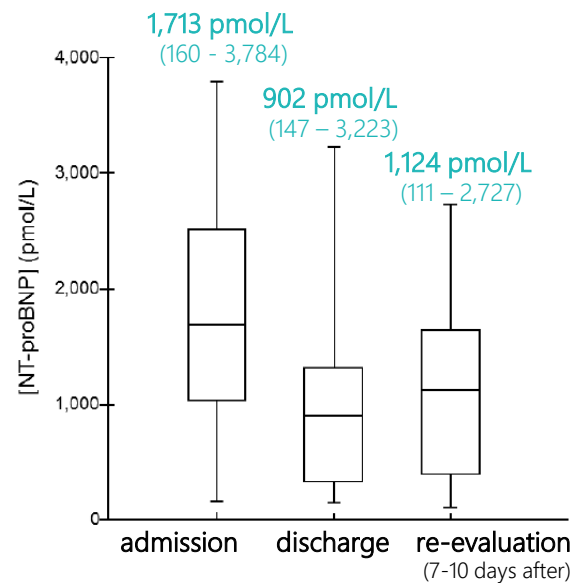
✓ NT-proBNP is not a stand-alone test

✓ NT-proBNP should be interpreted when used in conjunction with findings from the <u>history</u>, <u>physical examination</u>, <u>ECG</u>, auscultation, thoracic <u>radiography</u> and <u>echocardiography</u> ⇒ Helping achieve a definite diagnosis

Having a 'biomarker'-guided therapy may be very important as a complement to other testing in cats to improve the accuracy and confidence <u>in diagnosing heart disease</u> or <u>making treatment decisions</u> for cats with CHF.

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NT-proBNP in Cats

- Treatment Monitoring of NT-proBNP

1,713 pmol/L
(160 - 3,784)

902 pmol/L
(147 – 3,223)

1,124 pmol/L
(111 – 2,727)

[From Admission To Discharge]

Highly decreased NT-proBNP

⇒ Significantly longer survival time (182 days)

Mildly decreased NT-proBNP

⇒ Significantly shorter survival time (60 days)

Median NT-proBNP concentrations decreased significantly over the course of the 3 time points
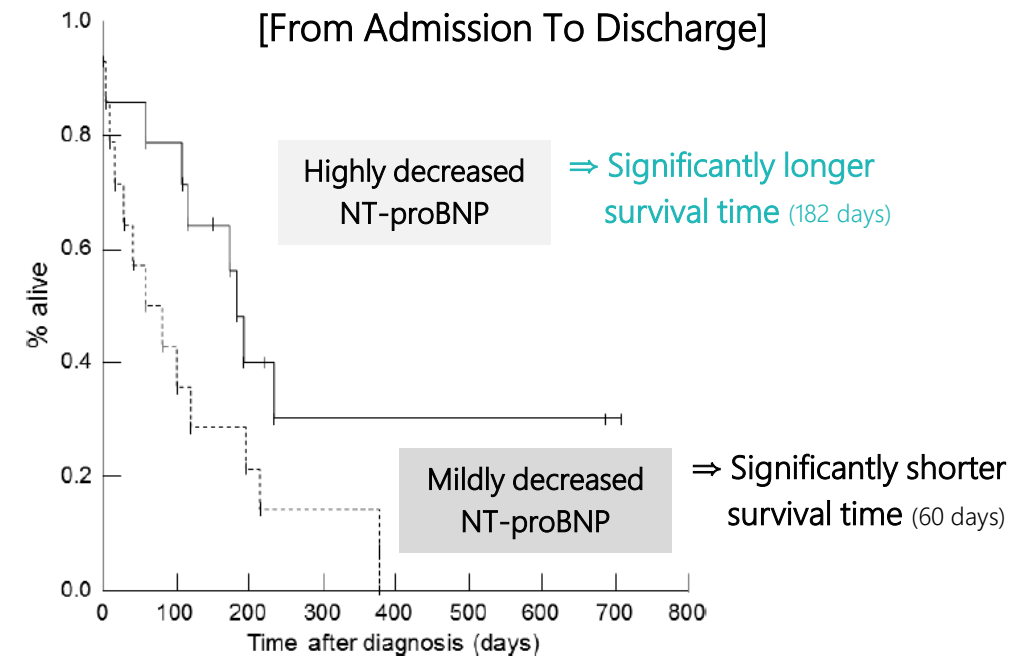
▲ Cats with CHF that had a decrease in the percent NT-proBNP concentration during hospitalization

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NT-proBNP in Cats
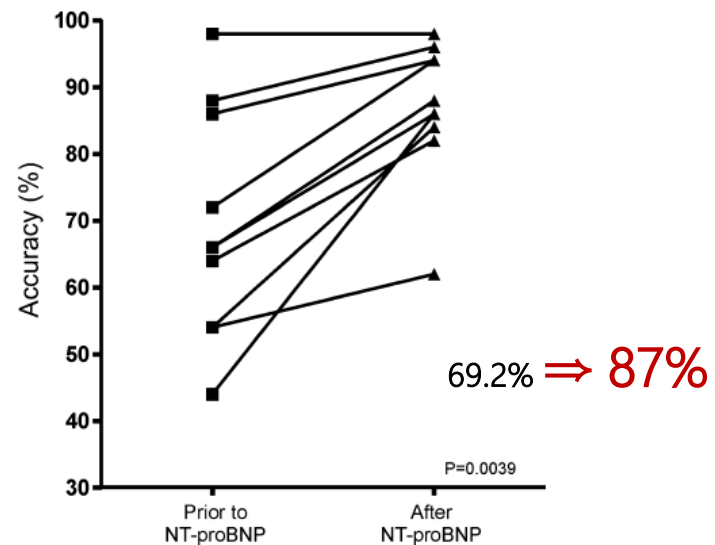
When NT-proBNP was measured,

More accurate the diagnosis is!

69.2% ⇒ 87%

Fig 1. Accuracy of general practitioners' diagnosis in cats with respiratory signs

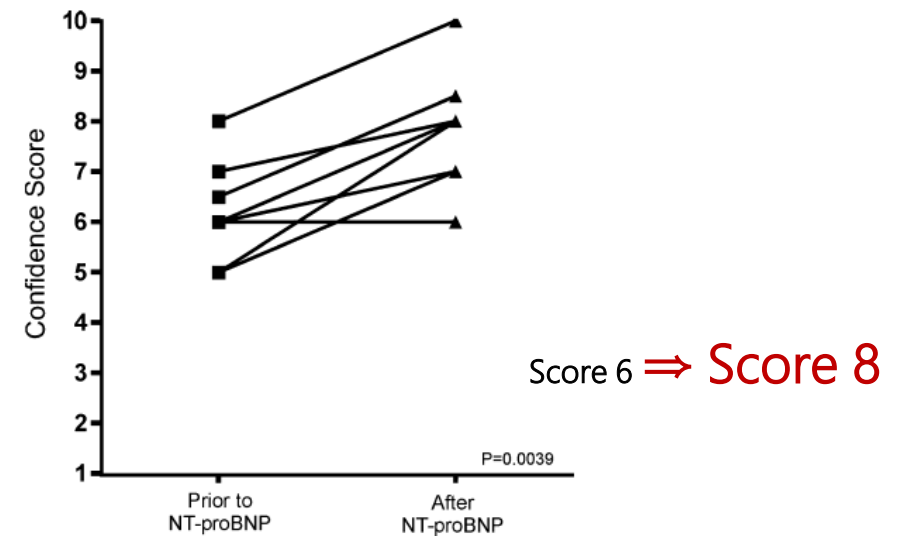Much higher the confidence score is!

Score 6 ⇒ Score 8

Fig 2. Confidence of general practitioners' diagnosis in cats with respiratory signs

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NT-proBNP in Cats

Factors which increase NT-proBNP concentrations

- Feline Hyperthyroidism

- Renal insufficiency or Pre-renal azotemia
  - ✓ NT-proBNP levels should be interpreted in the context of renal function tests.
  - ✓ Not significantly elevated in cats with mild to moderate (stages 1 to 3) CKD

- Systemic hypertension

- Severe arrhythmias

- Pulmonary hypertension (rare)

Elevated
NT-proBNP concentrations

+

Normal
Echocardiogram

↓

Consider these factors
which increase NT-proBNP levels!

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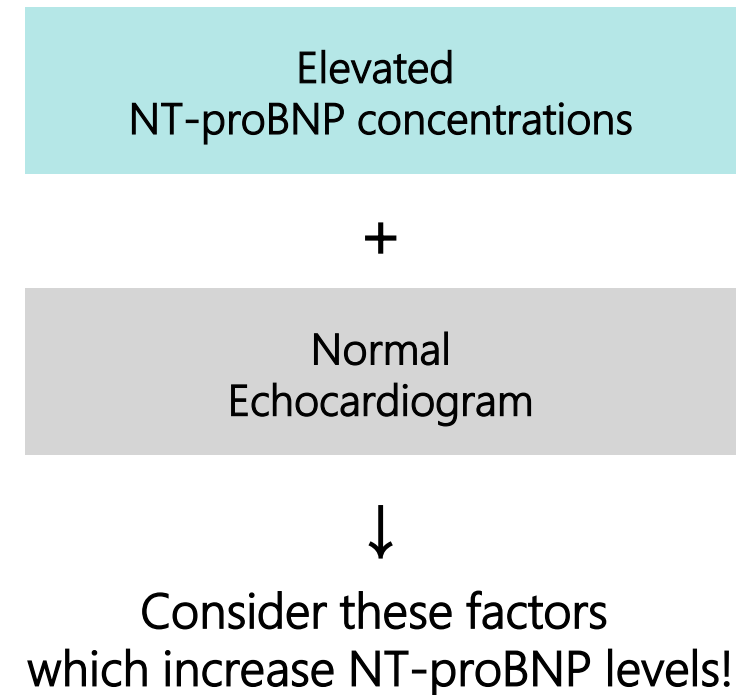
01 NT-proBNP

CASE STUDY -1

- **Patient information**

    - **Breed**: Korean short hair

    - **Age**: 7.6 yrs

    - **Sex**: Castrated male

- **History**

    - Long-term use of steroids due to stomatitis

    - No special abnormalities in the patient's appetite, vigor, etc.
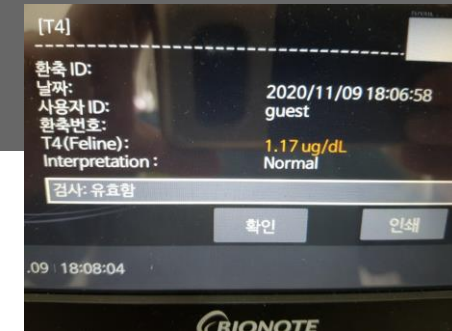
- **Chief complaint**

    - Visited the hospital for pre-anesthesia examination and NT-proBNP testing was conducted

    - SNAP Kits measured twice, all negative

    - Vcheck NT-proBNP: 1181.1 pmol/L (*normal: <100 pmol/L)

    ☞ Conducted an echocardiography and thyroid function test due to discrepancies between SNAP kit and Vcheck results


▲ NT-proBNP test results

▲ Vcheck T4 result

- **Test result**

    - **T4:** 1.17 ug/dl → FTH ruled out (*normal: 0.8~4.7 ug/dl)

    - **Echocardiography:** diagnosed with hypertrophic cardiomyopathy (HCM)

        ✓ Abnormal findings of the anatomical structure of the heart are not observed.

        ✓ Mitral and aortic valve normal findings without reverse flow findings

        ✓ Increased thickness of ventricular septum (> 7.5mm, normal < 5.9mm)

        ✓ Increased FS% (64%, normal < 45%)

        ✓ La:Ao ratio <1.37 (normal < 1.50)
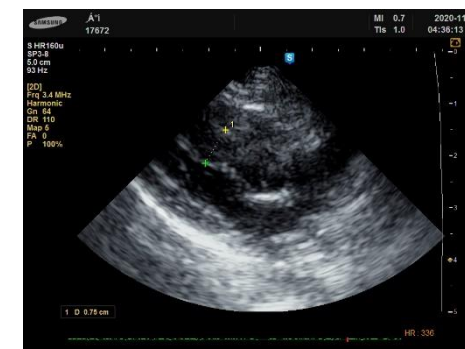
▲ IVSD=7.5mm
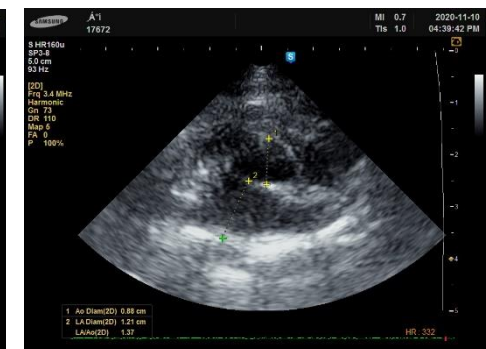
▲ La:Ao=1.37

CASE STUDY -2 (High NT-proBNP)

▲ 11 Y, Siamese, Castrated male

Signalment

- **Breed:** Siamese
- **Age:** 11 years old
- **Sex:** Castrated male
- **Body Weight:** 6.2 kg

Tests Performed

- Physical Examination
- Auscultation
- Thoracic radiograph
- Thoracic ultrasound
- NT-proBNP testing

Chief Complaint

- lethargy
- Inappetence
- Cough (Respiratory sign)

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CASE STUDY -2 (High NT-proBNP)

Test Results

- Physical Examination
    - BCS (Body condition score): 5
    - Heart rate: 172 (per minute)
    - Respiratory rate: 61 (per minute)
    - Auscultation: No murmur

- **NT-proBNP Testing**
    - **420 pmol/L**

- Thoracic ultrasound
    - LA : Ao 2.0 (< 1.5)
    - No pericardial effusion

▲ 11 Y, Siamese, Castrated male

Differential Diagnosis (Respiratory signs)

- Neoplasia (primary lung tumor, metastatic neoplasia)
- Idiopathic chylothorax
- feline asthma
- Fungal disease

Final Diagnosis

- Congestive Heart failure
- Hypertrophic cardiomyopathy (HCM)

CASE STUDY -3 (High NT-proBNP)

▲ 9M, Persian, male

Signalment

- **Breed:** Persian
- **Age:** 9 Months
- **Sex:** Male
- **B.W:** 2.8 kg

Chief Complaint

- Dyspnea 2 days after castration
- Hind limb paralysis

CASE STUDY -3 (High NT-proBNP)

Test Results

- Physical Examination
  - TPR: Normal
  - Auscultation: No murmur

- Thoracic radiograph
  - Alveolar pattern
  - Pulmonary edema

- Feline NT-proBNP: 1111 pmol/L

Final Diagnosis
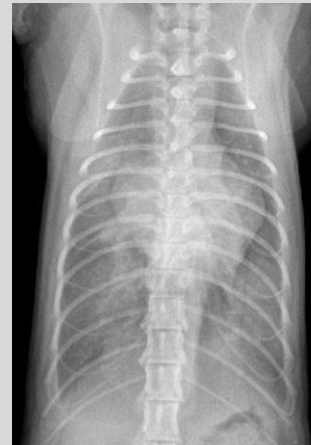 - Asymptomatic HCM
  - Died the next day

▲ 9M, Persian, male

◀ VD or DV view

▲ Lateral view (L)

▲ Lateral view (R)

L

CASE STUDY -4 (High NT-proBNP)

▲ 16.8Y, Mixed, female

Signalment

- **Breed:** Mixed
- **Age:** 16.8 years old
- **Sex:** Female

Physical Examination

- Lethargy
- Anorexia
- Dehydration

Chief Complaint

- lethargy
- Anorexia

CASE STUDY -4 (High NT-proBNP)

Other Test Results

- **Serum chemistry:** Increased BUN, Crea

- **Vcheck NT-proBNP:** 1097.1 pmol/L
- **IDEXX proBNP SNAP:** Strong positive

▲ 16.8Y, Mixed, female

Interpretation

➢ NT-proBNP concentrations can be elevated in cats with severe azotemia.

➢ Echocardiography should be performed to diagnosis a heart disease in this case.

Serum Chemistry/ Blood gas	Measured value	Reference range
T-pro	6.9	5.7–8.9 g/dL
Glu	90	74–159 mg/dL
BUN	>140	16–36 mg/dL
Crea	8.4	0.8–2.4 mg/dL
Na+	130	150–165 mmol/L
K+	2.9	3.5–5.8 mmol/L
Cl-	108	112–129 mmol/L
pH	7	7.310–7.462
Pco2	35.6	25–37
HCO3	8.9	14–22
Anion gap	16	<27

▲ Result of Serum chemistry / blood gas

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CASE STUDY -5 (High NT-proBNP)

- **Hospitalized**: pancreatitis, renal disease
- **Clinical signs**: Respiratory symptoms (2/24)

2/24		2/25		3/3

2/24 → **2/25** → **3/3**

- ✓ Showed respiratory signs
- ✓ **Thoracic Radiograph**: Pulmonary edema
- ⇒ **Diagnosis: Congestive Heart Failure**

- ✓ **Echocardiography**: LA/AO 2.0
- ✓ **NT-proBNP**: 1134.3 pmol/L

- ✓ **Respiratory symptoms recover**
- ✓ **Thoracic Radiograph**: lung is clear
- ✓ **NT-proBNP**: 514.7 pmol/L

CASE STUDY -5 (High NT-proBNP)

- Echocardiography (2/25)

M mode	Measured value	Reference range
LVDd/mm	14	15-18
LVDs/mm	7	6-10
LVPWd/mm	8	6-10
IVSd/mm	5	3-6
EPSS/mm	0.1	<0.5
AOD/mm	8	7-10
LAD/mm	17	7-13
LA/Ao	2.0	1-1.4
EF%	85	50-80
FS%	50	25-60

D mode	Measured value	Reference range
MV E m/s	1.5	<1
MV A m/s	1.5	<1
E/A (Mv)	1.0	0.9-1.3
TV E m/s	0.9	<1
TV A m/s	0.4	<1
E/A (Tv)	2.0	1-1.5
AO Vmax m/s	1.0	<1.7

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CASE STUDY -5 (High NT-proBNP)

- Blood Tests (including Serum chemistry)

	2/10	2/14	2/20	2/25	2/28	3/3	3/9	3/17	3/26
Creatinine (0.8 – 1.9 mg/dL)		3.0	3.0	3.33*		2.62*	3.1	2.6	2.32
BUN (20 – 30 mg/dL)		48	49	67*		46	47	55	61
Vcheck fPL	13.5	15.5	12.8	4.6			10.2	9.5	10.1
Vcheck fSAA					129.4	86.8		<5	<5
Vcheck NT-proBNP				1134.3		514.7	457.1	202.4	174.9

* Measured by blood gas (BUN: 15-34 mg/dl, Crea 1-2.21 mg/dL)
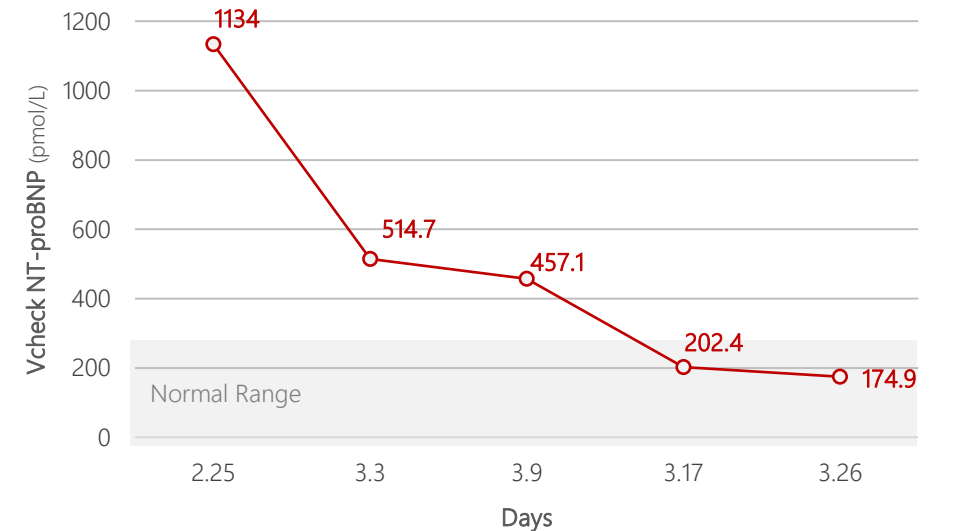
[NT-proBNP] Treatment Monitoring

01 NT-proBNP

CASE STUDY -6 (Normal case)

▲ 2Y, Munchkin, Female

Signalment

- **Breed:** Munchkin cat
- **Age:** 2 years old
- **Sex:** Female

Chief Complaint

- Vomiting
- Cough

Test Results

- **Thoracic Radiograph**
  - NSF

- **Echocardiography**
  - VHS 8.0 (6.9 - 8.1)
  - IVSd 5~5.2 (3 – 6)
  - LA/AO 1.03 (1 - 1.4)

- **EKG**
  - Normal

- **Abdominal Ultrasonography**
  - Normal gastrointestinal
  - Normal lymph nodes in the abdomen

- **Vcheck SAA**: <5 ug/ml

- **Vcheck NT-proBNP**: below 50 pmol/L

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CASE STUDY -7 (Normal case)

Signalment

- **Breed:** Stray cat
- **Age:** 6 years old
- **Sex:** Male

Chief Complaint

- For regular health check

Test Results

- **FeLV Ag/ FIV Ab:** (-) negative
- **Serum chemistry:** NSF
- **Vcheck NT-proBNP:** 59.9 pmol/L

Serum Chemistry	Measured value	Reference range
Alb	3.6	2.2–4.0 g/dL
T-pro	7	5.7–8.9 g/dL
Glu	116	74–159 mg/dL
ALP	50	14–111 U/L
T-bil	0.4	0.0–0.9 mg/dL
Amy	733	500–1500 U/L
BUN	22.6	16–36 mg/dL
Cre	0.9	0.8–2.4 mg/dL
Ca	10.5	7.8–11.3 mg/dL
PHOS	3.7	3.1–7.5 mg/dL
Na+	155	150–165 mmol/L
K+	4.4	3.5–5.8 mmol/L
GLOB	3.4	2.8–5.1 g/dL

Result of Serum chemistry ▶

02 Product Introduction

Vcheck Feline NT-proBNP

- Specifications

- Key Features

- Test Procedure

- Reference Range

- Performance

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Vcheck Feline NT-proBNP

- Specifications

  ✓ **Species** : Cat

  ✓ **Sample** : Serum 100 μl

  ✓ **Testing Time** : 10 minutes

  ✓ **Measurement** : Quantitative

  ✓ **Measurement Range** : 50 – 1,500 pmol/L

  ✓ **Storage Condition** : 1 - 30 °C

Vcheck Feline NT-proBNP

- Key Features

✓ **Quantitative measurement**

Quantifies the degree of elevation in NT-proBNP for an accurate evaluation

✓ **High correlation with laboratory ELISA**

Vcheck Feline NT-proBNP has a high correlation (R² = 0.96) with company 'I' laboratory.

✓ **A wide range of measurement**

Measures up to 1500 pmol/L

✓ **A user-friendly procedure & Fast results**

Simple one-step procedure, improving user convenience, and quick results within 10 min.

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Vcheck Feline NT-proBNP

- Test Procedure

✓ Samples should be centrifuged and tested immediately after collection. Alternatively, refrigerate and use within 24 hours or freeze.

  * Degradation of NT-proBNP may occur if stored at room temperature or refrigerated for more than 24 hours, causing false negative results.
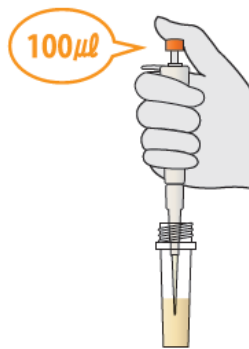
Add 100 µl of the sample to the assay diluent tube

*Stored samples should be placed at room temperature 30 min. before use.

Mix well by using a 100 µl pipette
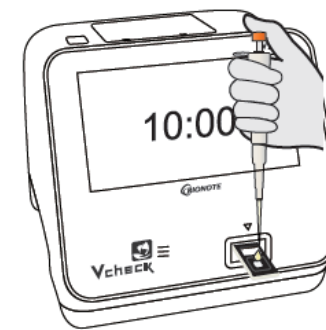
Add the mixed sample (100 µl) into the test device
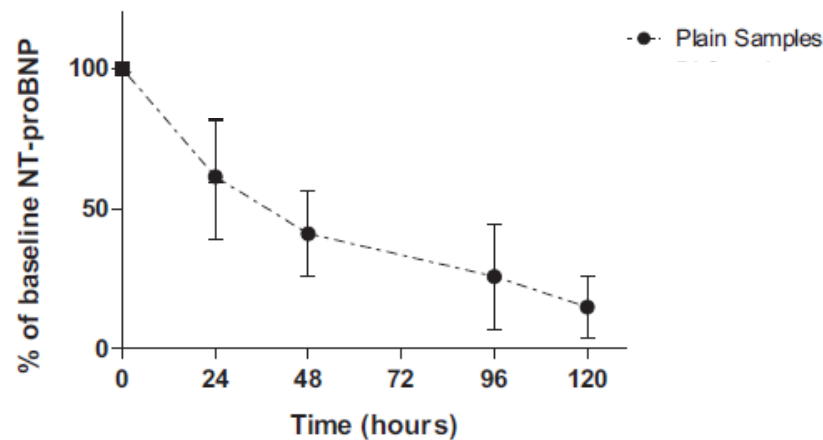
BIONOTE

Vcheck Feline NT-proBNP

- ### Stability of Feline NT-proBNP

  NT-proBNP: peptide hormone $\Rightarrow$ degradation of NT-proBNP by protease
    - Enzymatic activity is temperature dependent, decreasing at lower temperature.
    - Significant serum and plasma NT-proBNP degradation occurred at room temperature or under refrigeration (more than 1 day)
    - Therefore practitioners should be encouraged to process samples as quickly as possible. Alternatively, refrigerate and use within 24 hours or freeze.
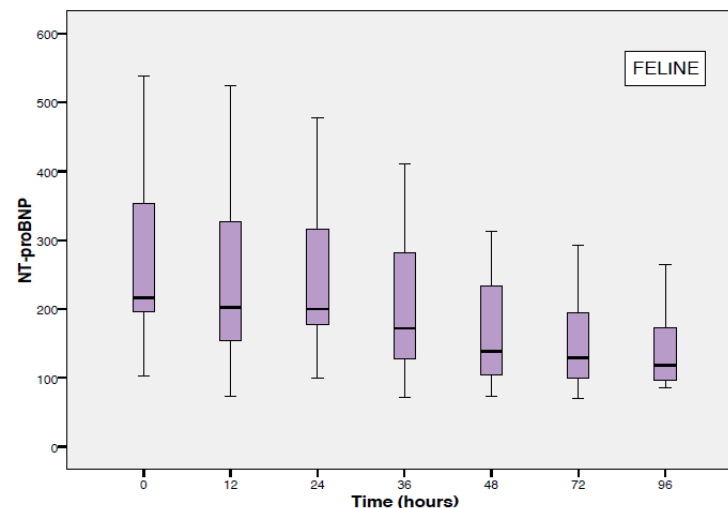
At room temperature,
38% decrease after 24 hours

Feline plasma stored in ice packs,
There was a significant difference at all time points, except at 12 hours

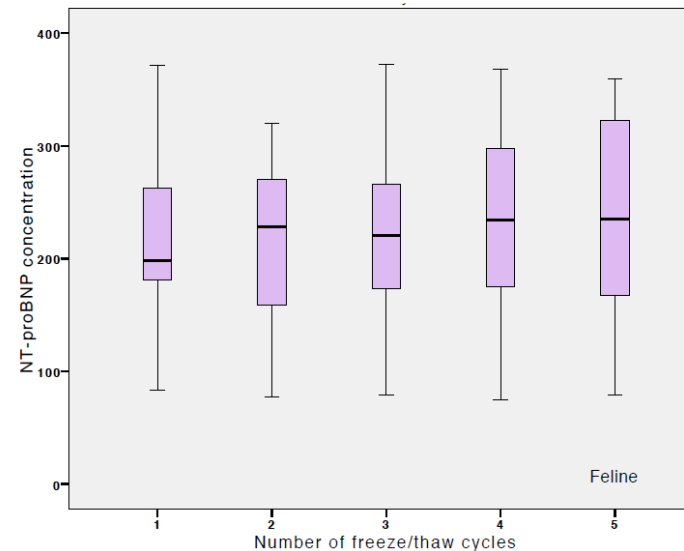## Vcheck Feline NT-proBNP

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  - Significant serum and plasma NT-proBNP degradation occurred at room temperature or under refrigeration (more than 1 day)
  - Therefore, practitioners should be encouraged to process samples as quickly as possible. Alternatively, refrigerate and use within 24 hours or freeze.

Over five freeze/thaw cycles,
Median NT-proBNP in feline plasma
did not change significantly.

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Vcheck Feline NT-proBNP

- Reference Range

| < 100 pmol/L	≥ 100 pmol/L
Normal	Abnormal Additional diagnostics are recommended

- A positive NT-proBNP test result should always be interpreted in combination and other diagnostic findings.

- In cats with respiratory signs, if the NT-proBNP is >270 pmol/L, CHF is the most likely cause of the clinical signs.

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Vcheck Feline NT-proBNP

- Performance

Comparative evaluation of feline NT-proBNP (N=37)

$y = 1.0211x + 8.4432$
$R^2 = 0.9645$
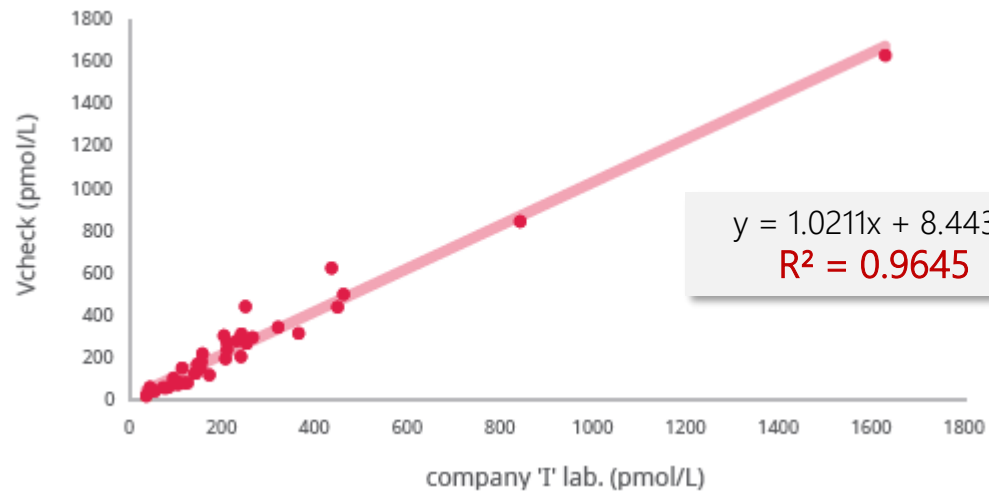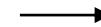
- Reproducibility

  CV < 15%

- Accuracy

  Bias < 20%

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Feline NT-proBNP: Competitor's product

- Point-of-care 'S' kit of company 'I' − Qualitative analysis

'S' kit of company 'I'	Normal	Abnormal	
	Sample spot is lighter than reference spot.	Sample spot is the same color as reference spot.	Sample spot is darker than reference spot.
Company 'I' laboratories	<150 pmol/L	150–200 pmol/L	>200 pmol/L

Comparison of the color intensity of 'S' Feline proBNP Test's sample spot to potential Cardiopet proBNP Test results

- Qualitative measurement
- Cannot quantifies the concentrations NT-proBNP for an accurate evaluation
- Cannot tell the exact value of cut-off

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Reference

Reference

1. Mark Oyama. Cardiac Blood Tests in Cats: Another Tool for Detection of Heart Disease. Today's Veterinary Practice. September/October 2011
2. Natalie Stilwell, MVC 2018: Advances in Feline Heart Disease Diagnosis
3. Connolly DJ, Soares Magalhaes RJ, Fuentes VL, et al. Assessment of the diagnostic accuracy of circulating natriuretic peptide concentrations to distinguish between cats with cardiac and non-cardiac causes of respiratory distress. J Vet Cardiol 2009;11(Suppl 1):S41–S50
4. K.V. Pierce, J.E. Rush, V.K. Yang, et al. Association between Survival Time and Changes in NT-proBNP in Cats Treated for Congestive Heart Failure. J Vet Intern Med. 2017 May-Jun; 31(3): 678-684.
5. Dr. Sonya G Gordon. Cardiac Education Group. October 2014
6. Fox PR, Rush JE, Reynolds CA, et al. Multicenter evaluation of plasma N-terminal pro-brain natriuretic peptide (NT-pro BNP) as a biochemical screening test for asymptomatic (occult) cardiomyopathy in the cat. J Vet Intern Med 2011; in press.
7. Connolly, DJ , et al. The effect of protease inhibition on the temporal stability of NT-proBNP in feline plasma at room temperature. J Vet Cardiol 2011;13:13–19.

Product No.	Product Name	Product Type	Packing Unit
VCF130DC	Vcheck Feline NT-proBNP	Device	5 Tests/Kit

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Thank you

BIONOTE Marketing team

Mar. 2020

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