Quantitative marker of myocardial injury, Troponin I

Vcheck Feline TnI

BIONOTE Marketing team

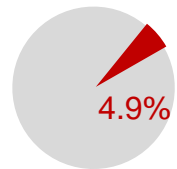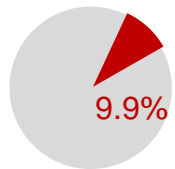DEC 2021

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Hypertrophic Cardiomyopathy (HCM)

The most common heart disease, one of the 10 most common causes of death in cats

- **Prevalence[1]**:15% in the general cat population, up to 29% in older cats (<u>In apparently healthy cats</u>)

4.9%	9.9%	18.6%	29.4%
Juvenile (6-12m)	Young (1-3 years)	Adult (3-9 years)	Senior (≥9 years)
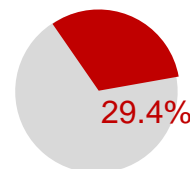
- **Clinical signs**: no symptoms, labored breathing

- **Diagnosis**: Echocardiography; LV wall ≥ 6 mm
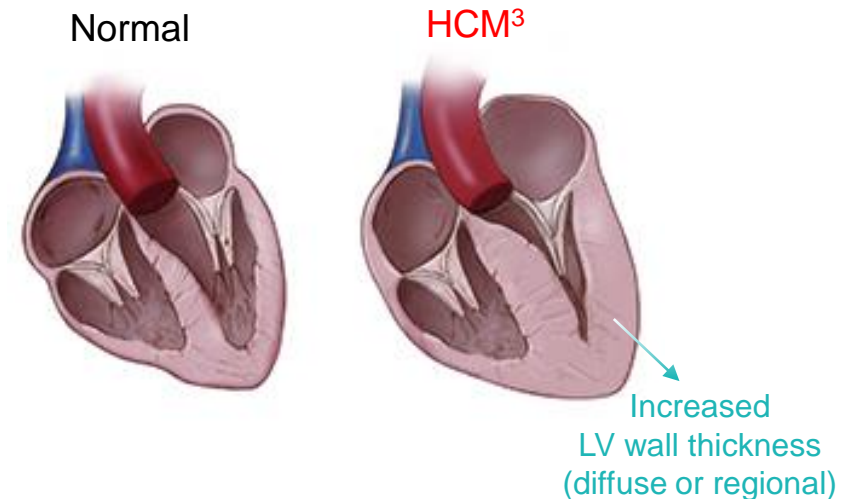
Normal

HCM[3]

Increased
LV wall thickness
(diffuse or regional)

Reference: 1. J Vet Cardiol. 2015 Dec;17 Suppl 1:S244-57. 2. image; https://www.vet.cornell.edu/departments-centers-and-institutes/cornell-feline-health-center/health-information/feline-health-topics/hypertrophic-cardiomyopathy

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Hypertrophic Cardiomyopathy (HCM)

The most common heart disease, one of the 10 most common causes of death in cats

- **Complications**:

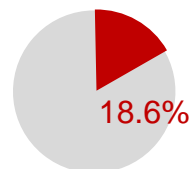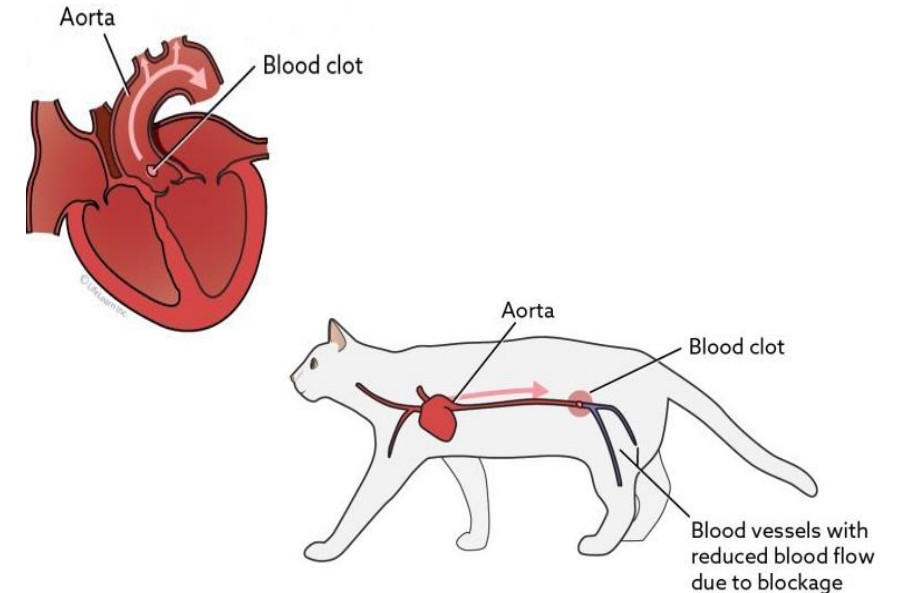 ① **Congestive heart failure (CHF)** 28% of HCM cats[2]
  : The increase in pressure in the left atrium causes back pressure in the blood vessels of the lungs and a fluid build up in the lungs (tachypnea, labored breathing)

  ② **Arterial thromboembolism (ATE)** 12-21% of HCM cats[3]
  : The clot, which is formed in the left atrium, travels to an artery and becomes an ATE, which then blocks the blood flow, causing infarction. (paralysis, severe pain)

- **Mortality**[4]: 23% (cardiovascular death within 5 years)

Aorta
Blood clot

Aorta
Blood clot
Blood vessels with reduced blood flow due to blockage

Reference: 1. image: https://vcahospitals.com/know-your-pet/aortic-thromboembolism-in-cats 2. Frontiers in Veterinary Science, 6. doi:10.3389/fvets.2019.00015 3. Open Veterinary Journal, (2020), Vol. 10(3): 340–346. 4. J Vet Intern Med. 2018 May;32(3):930-943

Feline Heart Disease: HCM

Diagnosis of HCM

- Auscultation

Not sensitive and specific
(up to 80% in HCM cats,
 30-45% of healthy cats)

- ECG (Electrocardiogram)
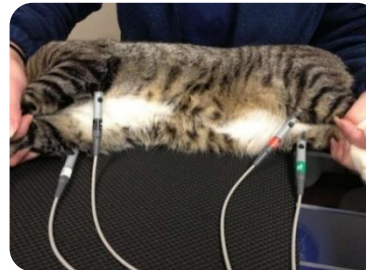
Not recommended

- X-ray (Radiography)

CHF

Insensitive for mild or moderate HCM,
but the gold standard for the presence of CHF

- Echocardiography

HCM

Only gold standard test for diagnosis of HCM,

Reference: 1. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. J Vet Intern Med. 2020;1–16.

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Feline Heart Disease: HCM
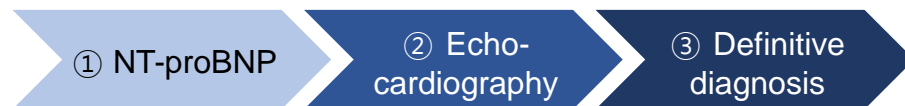
NT-proBNP: "ACVIM consensus statement guidelines"

- The measurement of NT-proBNP can be considered **as an initial screening test** for identifying advanced HCM.

- When X-ray or echocardiography are unavailable, evaluation of NT-proBNP concentrations should be considered.

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Abstract
Cardiomyopathies are a heterogeneous group of myocardial disorders of mostly unknown etiology, and they occur commonly in cats. In some cats, they are well-tolerated and are associated with normal life expectancy, but in other cats they can result in congestive heart failure, arterial thromboembolism or sudden death. Cardiomyopathy classification in cats can be challenging, and in this consensus statement we outline a classification system based on cardiac structure and function (phenotype). We also introduce a staging system for cardiomyopathy that includes subdivision of cats with subclinical cardiomyopathy into those at low risk

In apparently healthy cats,

The measurement of NT-proBNP can be considered as an initial screening test.

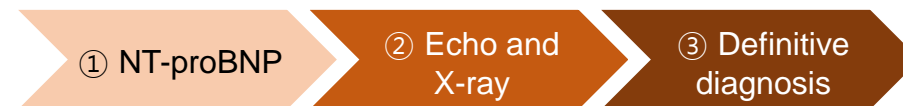① NT-proBNP → ② Echo-cardiography → ③ Definitive diagnosis

≥ 100 pmol/L
: High possibility of HCM

< 100 pmol/L
: Normal results do not assure that a cat is free of HCM

In cats with respiratory signs,

Point-of-care NT-proBNP test should be considered (high level of evidence).

① NT-proBNP → ② Echo and X-ray → ③ Definitive diagnosis

≥ 270 pmol/L
: CHF is more likely to be the cause

< 270 pmol/L
: Respiratory disease is more likely to be the cause

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Reference: 1. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. J Vet Intern Med. 2020;1–16.
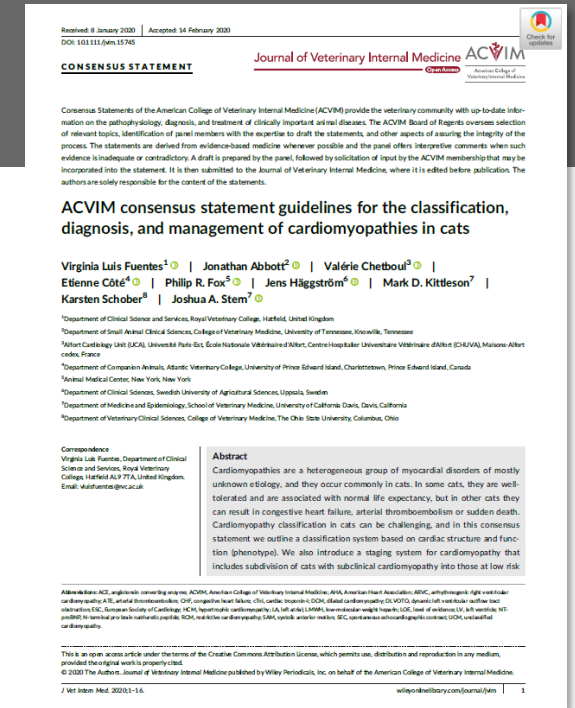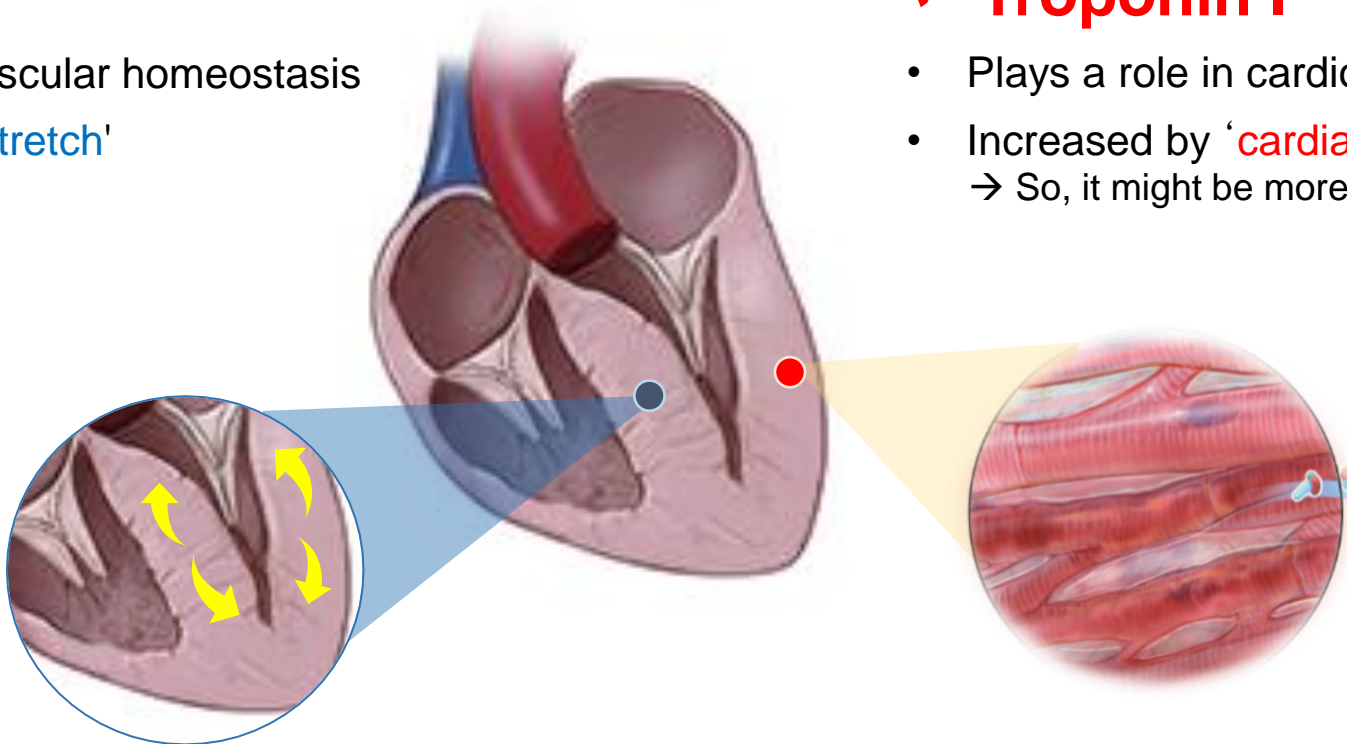
Cardiac Biomarkers

Two Useful Cardiac markers in Cats

✓ **NT-proBNP**

- Plays a role in cardiovascular homeostasis
- Increased by 'cardiac stretch'

✓ **Troponin I**

- Plays a role in cardiomyocyte contraction
- Increased by 'cardiac injury itself'
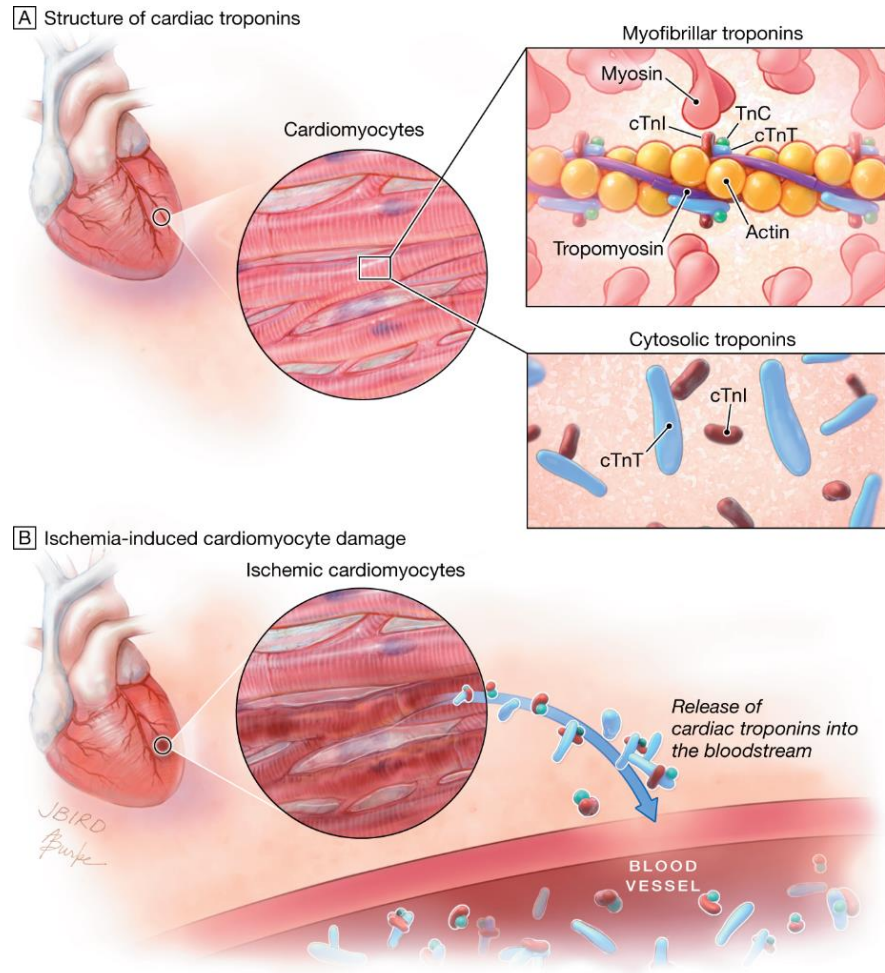 → So, it might be more suitable in cats with HCM

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Cardiac Biomarkers

Two Useful Cardiac markers in Cats

Indication	NT-proBNP	Troponin I
Screens for HCM in apparently healthy cats	◯[1,2]	◯[7,8]
Discriminates between cardiac and non-cardiac causes of respiratory distress	◯[3.4]	△[9]
Evaluates increased risk of cardiovascular death	◯[5]	◯[10]
Differentiates grades of severity of HCM	◯[6]	◯[8]

Reference: 1. J Vet Cardiol. 2014; 16:245-255. 2. J Vet Intern Med. 2017;31:994-999. 3. J Vet Cardiol. 2009;11(Suppl 1):S51-S61. 4. J Vet Intern Med. 2012;26:542-546. 5. J Vet Intern Med 2017;31:678–684. 6. Vet Clin Pathol. 2011 Jun;40(2):237-44. 7. J Vet Intern Med. 2018;32:922-929. 8. J Vet Intern Med. 2019;33:1242-1250. 9. J Am Vet Med Assoc. 2008;233:1261-1264. 10. J Vet Intern Med. 2014;28:1731-1737.

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Troponin I

A Structure of cardiac troponins

Myofibrillar troponins

Cardiomyocytes

Myosin

cTnI — TnC — cTnT

Tropomyosin — Actin

Cytosolic troponins

cTnI

cTnT

B Ischemia-induced cardiomyocyte damage

Ischemic cardiomyocytes

Release of cardiac troponins into the bloodstream

BLOOD VESSEL

JBIRD Burke

✓ After cardiac insult, a rise of **Troponins** can be seen within 2-3 hours, and peak concentration is frequently reached in 18-24 hours.

✓ In human medicine, Troponin I (TnI) is used for the diagnosis of acute myocardial infarction (AMI).

In cats,

TnI is useful for assessing hypertrophic cardiomyopathy (HCM), which is the most common heart disease.

1) **Screening for HCM in apparently healthy cats**

2) **Predictor of cardiac death in cats with HCM**

Reference: 1. JAMA. 2013;309(21):2262-2269. 2. J Vet Intern Med. 2016 Jan-Feb; 30(1): 36–50.

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Troponin I

1) Screening for HCM in apparently healthy cats

"Troponin I might be considered for differentiating between normal cats and cats with subclinical HCM when cardiac disease is suspected"[1] (Level of evidence: medium)

- Troponin I reflects **myocardial damage** as a consequence of developing HCM.

- If other causes of cardiac injury have been ruled out, measuring TnI provides additional information that is useful for assessing the severity of HCM.

  - **TnI < 0.163 ng/ml** : Likely excludes HCM

  - **TnI > 0.234 ng/ml** : Likely identifies severe HCM
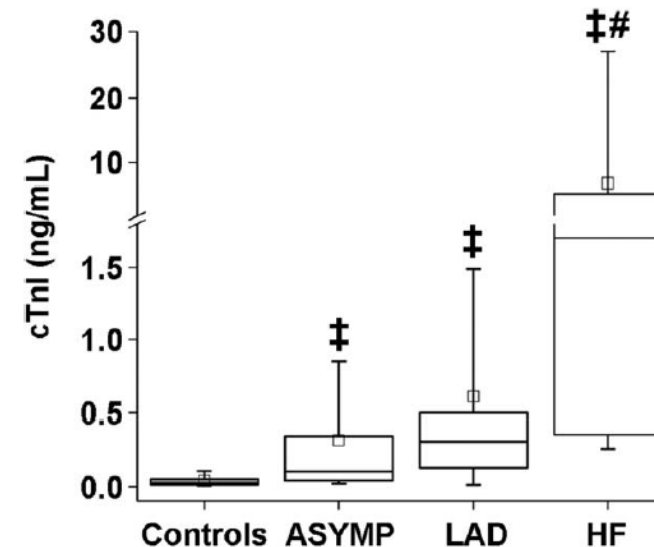
ASYMP: asymptomatic HCM without LA dilatation, **LAD**: asymptomatic HCM with LA dilatation, **HF**: cats with heart failure

Reference: 1. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. J Vet Intern Med. 2020;1–16. 2. J Vet Intern Med. 2018 May;32(3):922-929.
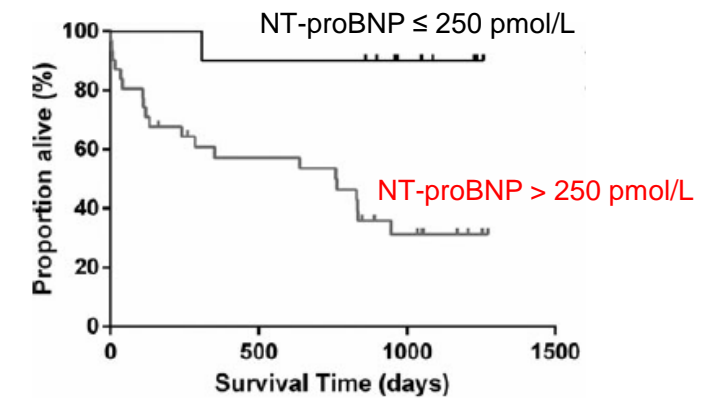
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Troponin I

2) Predictor of cardiac death in cats with HCM

"An increased TnI concentration is associated with increased risk of cardiovascular death"[1] (Level of evidence: high)

- **Poor prognosis of HCM cats**: CHF, ATE, increased LA size …

- (In humans) a single measurement of NT-proBNP, TnI, or a combination of both, act as independent predictors of adverse cardiovascular events.

- Predictors of cardiac death in cats with HCM

  - **NT-proBNP**: cut-off 250 pmol/L → Hazard ratio 10

  - **Troponin I**: cut-off 0.7 ng/ml → Hazard ratio 5
  (independent of the presence of CHF or LA dilatation)

Reference: 1. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. J Vet Intern Med. 2020;1–16. 2. J Vet Intern Med. 2014;28:1731-1737.
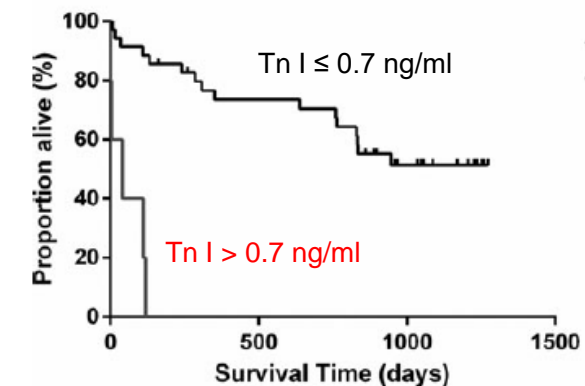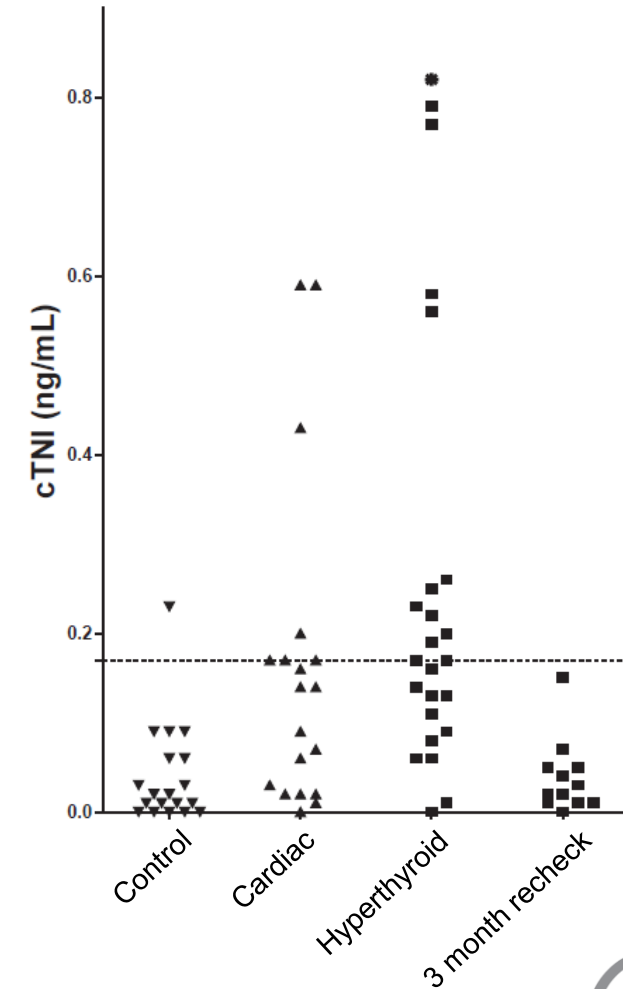
Conditions affecting TnI concentrations

Troponin I measurement detects only myocardial injury.

Non-cardiac disease also might cause **secondary myocardial injury**.

- **Hyperthyroidism[1]** (In old cats)
- **Renal disease[2]**
- **Respiratory disease[3]**
- **Anemia[4]**
- **Neoplasia[5]**

Reference: 1. J Vet Intern Med 2014;28:465–472.   2. J Feline Med Surg. 2019 Oct;21(10):985-991.   3. J Am Vet Med Assoc 2014;244:425–430.   4. J Small Anim Pract. 2014 Jun;55(6):320-2.   5. Vet J 2010;185:50–57.

Product Introduction
Vcheck Feline TnI

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Product Introduction

Vcheck Feline TnI

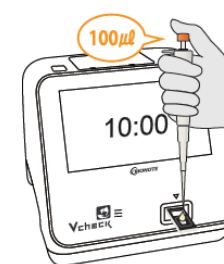*Quantitative marker of myocardial injury*

1. Add 100 µl of the sample to the assay diluent tube
2. Mix well 5-6 times by using a 100 µl pipette
3. Add 100 µl of the mixed sample into the test device
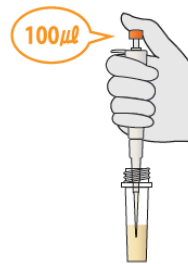
Samples should be tested immediately after collection.

(If not, freeze the samples at -20 °C or below for storage. Do not freeze and thaw repeatedly.)

- **Sample**: Serum 100 µl
- **Testing Time**: 10 minutes
- **Measurement** : Quantitative
- **Measurement Range**: 0.01 – 20 ng/ml
- **Storage Condition**: 1 - 30 ℃

* TnI concentrations should not be used to either confirm or exclude primary cardiac disease without the simultaneous use of echocardiography.

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Product Introduction

Vcheck Feline TnI

Quantitative marker of myocardial injury

Apparently healthy cats with cardiac risk factors

| Troponin I			NT-proBNP	
< 0.16 ng/ml	0.16 – 0.23 ng/ml	> 0.23 ng/ml	< 100 pmol/L	≥ 100 pmol/L
Heart disease not likely	Heart disease likely	Severe heart disease likely	Heart disease not likely	Heart disease likely

- **Sample**: Serum 100 μl
- **Testing Time**: 10 minutes
- **Measurement** : Quantitative
- **Measurement Range**: 0.01 – 20 ng/ml
- **Storage Condition**: 1 - 30 ℃

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Performance

Correlation

Vcheck Feline TnI has a strong correlation (y=0.94x-0.0442, **R²=0.9896** in entire concentration; y=1.012x-0.0636, **R²=0.9684** in low concentration) with the ECLIA method from 'R' multinational healthcare company.

Entire concentration (0~20 ng/ml)

$$y = 0.94x - 0.0442$$
$$R^2 = 0.9896$$

N=234
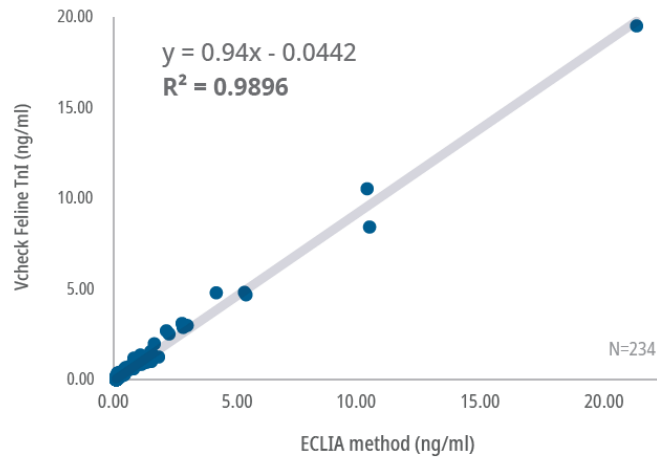
Low concentration (0~6 ng/ml)

$$y = 1.012x - 0.0636$$
$$R^2 = 0.9684$$

N=231
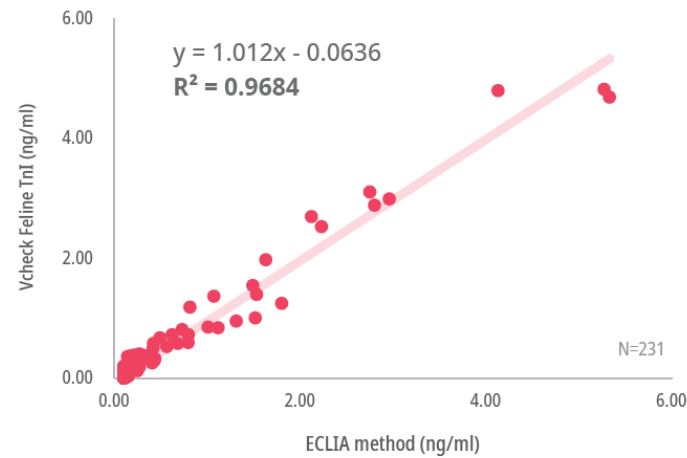
Comparative analysis of TnI		Roche (ng/ml)		Total
		> 0.12	≤ 0.12	
Vcheck (ng/ml)	> 0.12	46	8	**54**
	≤ 0.12	5	175	**180**
Total		**51**	**183**	**234**

- **Sensitivity 90.2%** (46/51)
- **Specificity 95.6%** (175/183)

- **Accuracy 94.4%** (221/234)

*Internal Evaluation Data

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Performance

Precision

In the repeatability tests (within run, between run, between tester, between day and between lot), all test results were confirmed to meet the acceptance criteria.

Concentration	Criteria
Level 1 (Low)	SD ≤ 0.3 ng/ml
Level 2 (Medium)	CV ≤ 15%
Level 3 (High)	CV ≤ 15%

Interference

No interference was observed for each substance up to the concentration presented in the following table.

Interfering substances	Concentration
Hemoglobin	< 150 mg/dL
Intralipid	< 2,500 mg/dL
Cholesterol	< 250 mg/dL
Bilirubin	< 20 mg/dL
Vitamin C	< 100 mg/dL

* Moderate or Severe hemolysis can falsely increase TnI levels.

Cross-reactivity

There was no cross-reactivity with skeletal muscle troponin I, cardiac troponin T and troponin C (200 ng/ml, respectively).

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