

Catalyst proBNP Test: an accurate and reliable in-house quantitative NT-proBNP assay for dogs and cats

Introduction

Heart disease is a major cause of ill-health in both dogs and cats. Approximately 10% of dogs are estimated to have heart disease with myxomatous mitral valve disease (MMVD) being the most common canine cardiac disorder diagnosed.¹ In cats, hypertrophic cardiomyopathy (HCM) is the most common cause of heart disease, with an estimated prevalence of 15% in the general cat population.² Although echocardiography performed by a veterinary cardiology specialist is widely considered to be the diagnostic test of choice for MMVD and feline cardiomyopathies,^{1,2} establishing a definitive diagnosis of heart disease in companion animals can be challenging. Access to specialist echocardiography is not possible for many cases, and so the potential of biomarkers to assess cardiac health in a minimally invasive, cost-effective manner has been intensively explored over the past two decades.^{3,4}

B-type natriuretic peptide (BNP) is stored in cardiomyocytes as a prohormone (proBNP) and is released in response to stretch or strain on heart muscle.³ On release, proBNP is cleaved into a biologically active hormone C-BNP and an inactive amino-terminal fragment (NT-proBNP). NT-proBNP has a longer half-life than C-BNP, making the quantification of circulating NT-proBNP more amenable for clinical purposes. NT-proBNP is released in proportion to the amount of stretch and strain on the myocardium. IDEXX currently offers quantification of NT-proBNP in both canine and feline EDTA plasma samples and feline serum samples through the IDEXX Reference Laboratories Cardiopet™ proBNP Test^{5,6} as well as the semiquantitative, point-of-care SNAP™ feline proBNP Test.^{7,8}

The measurement of NT-proBNP is informative in the three key clinical presentations. Firstly, the measurement of NT-proBNP can be used to screen cats as part of annual wellness or preanaesthetic checks for subclinical heart disease.^{9,10} Secondly, NT-proBNP concentrations can help to assess whether a healthy, small- to medium-breed dog with a heart murmur has stage B1 or B2 heart disease.^{11,12} Thirdly, the quantification of NT-proBNP can be used to help to diagnose the cause of respiratory distress in both dogs^{13,14} and cats.^{15–17} The measurement of NT-proBNP can also be helpful in a wide range of other clinical scenarios, such as screening at-risk dog breeds for dilated cardiomyopathy¹⁸ and in the assessment of ascites in dogs.¹⁹ The quantification of NT-proBNP can also provide insights into the response of therapy and longer-term prognosis in both dogs^{20–22} and cats.^{23,24}

The Catalyst™ proBNP Test is an immunologic assay that is specifically designed to align with the Cardiopet proBNP Test to provide quantitative NT-proBNP results at the point-of-care for dogs and cats. The same Catalyst proBNP Test can be used to quantify NT-proBNP in either feline or canine serum or lithium heparin plasma samples. The assay has a wide dynamic range (canine: 250–10,000 pmol/L; feline: 24–1,500 pmol/L) and provides results within 16 minutes. Having the ability to quantify NT-proBNP accurately and reliably during the patient visit enables timely, in-person communication with pet owners. This not only supports shared decision-making but may also enhance client understanding and adherence to subsequent diagnostic and treatment recommendations.

This study aimed to compare the performance of the Catalyst proBNP Test to the Cardiopet proBNP Test, assess the precision of the Catalyst proBNP Test, and evaluate the influence of common interfering substances.

Materials and methods

Method comparison

A method comparison study was conducted to evaluate the accuracy of the Catalyst proBNP Test. The study used serum samples originally collected and submitted to IDEXX Reference Laboratories for Cardiopet proBNP testing. The samples were frozen after testing at the laboratory and then thawed for analysis on both the Catalyst proBNP and Cardiopet proBNP tests at the same time. A single replicate of the Cardiopet proBNP Test served as the reference standard for comparison. Depending on the sample volume available, two to five Catalyst proBNP replicates were used to generate the mean NT-proBNP concentration used in the analysis.

Correlation and bias between the Catalyst proBNP Test and the Cardiopet proBNP reference method was assessed using Passing–Bablok regression and Pearson's correlation coefficient. Concordance with interpretative zone (low/mid/high) was also assessed. All method comparison analyses were done as per CLSI EP09c guidelines.

Precision

Precision was assessed per CLSI EP05-A3 guidelines using pooled canine and feline serum samples at three NT-proBNP concentrations, as shown in table 2; repeatability, the precision component most relevant to routine assay use, is reported here. Testing was

performed over three consecutive days on two Catalyst Dx™ and four Catalyst One™ chemistry analyzers, with five replicate measurements obtained from each analyzer per day.

Interfering substances

Pooled canine and pooled feline serum samples with low (canine: 320 pmol/L; feline: 90 pmol/L) and high (canine: 1,300 pmol/L; feline: 320 pmol/L) NT-proBNP concentrations and visually free of interfering substances were prepared for interference testing. For canine samples, exogenous NT-proBNP was added to create the high canine sample pool. For feline samples, endogenously high NT-proBNP samples were used. To assess the potential impact of common interferent (hemolysis, lipemia, and icterus), canine or feline red blood cell hemolysate, intralipid, and ditaurobilirubin were spiked at varying concentrations into the samples, as detailed in table 3. Each sample was analyzed on six Catalyst One and six Catalyst Dx analyzers and the results reported are the mean of the 12 replicates. Results flagged as potential outliers by Rosner's extreme Studentized deviate (ESD) test were excluded.

Sample type comparison

Paired canine and feline serum and plasma samples from the same patients were tested on the Catalyst proBNP Test and compared using a paired *t* test, with agreement also assessed by interpretative zone (low/mid/high) concordance.

Results

Method comparison

A regression plot evaluating correlation between mean Catalyst proBNP and Cardiopet™ proBNP test results across the dynamic range is shown in 205 canine (figure 1) and 219 feline (figure 2) samples. For canine samples, the Passing-Bablok regression had a Pearson's correlation coefficient of $r = 0.95$, with a slope of 0.92 (95% CI: 0.88–0.97) and an intercept of 12.9 (95% CI: -32.1, 48.3). The wide intercept confidence interval observed for the canine dataset reflects extrapolation of the regression line to a concentration of zero, well outside the reportable range of the assay, rather than a lack of precision in the fitted slope. For feline samples, the correlation coefficient was $r = 0.98$, with a slope of 0.95 (95% CI: 0.93, 0.98) and an intercept of -3.1 (95% CI: -6.6, 2.2).

Categorizing results by standard NT-proBNP interpretative cutoffs, the two methods agreed on classification in the large majority of cases (table 1), with most discordant pairs falling into adjacent categories rather than across wide ranges.

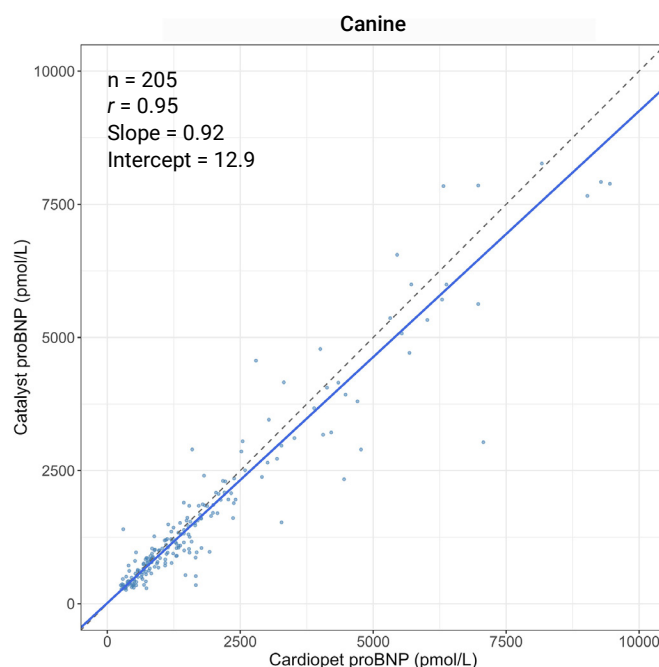


Figure 1. Correlation graph of pairwise comparisons of Catalyst proBNP and Cardiopet proBNP tests in canine samples. The line of best fit is shown on the graph (solid blue). $X = Y$ is shown as a gray dashed line.

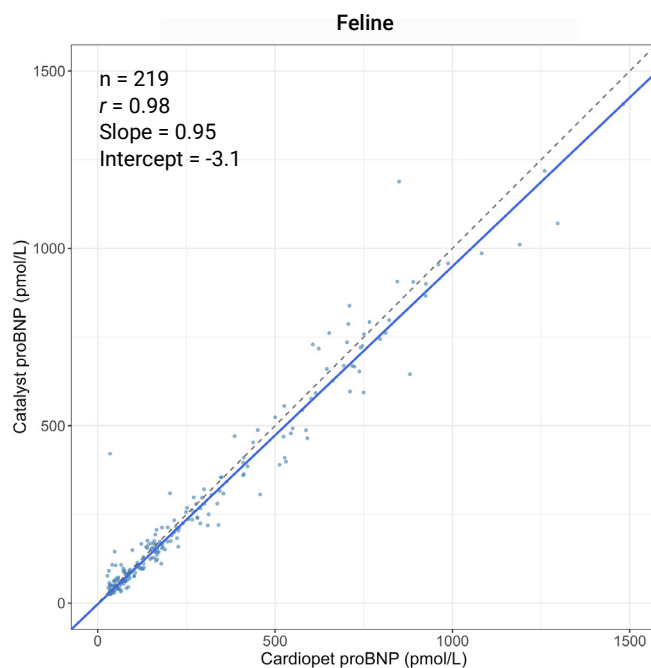


Figure 2. Correlation graph of pairwise comparisons of Catalyst proBNP and Cardiopet proBNP tests in feline samples. The line of best fit is shown on the graph (solid blue). $X = Y$ is shown as a gray dashed line.

Species	Cardiopet™ proBNP Test				
Canine	Catalyst™ proBNP Test		< 900 pmol/L	900–2,500 pmol/L	> 2,500 pmol/L
		< 900 pmol/L	73	13	0
		900–2,500 pmol/L	7	72	3
		> 2,500 pmol/L	0	2	35
Feline	Catalyst™ proBNP Test		< 100 pmol/L	100–270 pmol/L	> 270 pmol/L
		< 100 pmol/L	66	9	0
		100–270 pmol/L	7	60	8
		> 270 pmol/L	1	1	67

Table 1. Canine and feline contingency table. Canine n = 205, overall concordance = 87.8%. Feline n = 219, overall concordance = 88.1%.

Precision

The Catalyst proBNP Test precision study results, based on a three-day study design, are summarized in table 2. The assay demonstrated a repeatability coefficient of variation (%CV), that ranged from 10.77% to 13.68% across clinically relevant canine NT-proBNP concentrations (798.7–2,822.8 pmol/L). The assay demonstrated a repeatability coefficient of variation (%CV) that ranged from 8.58% to 16.87% across clinically relevant feline NT-proBNP concentrations (88.9–327.9 pmol/L).

Species	Level	Mean concentration (pmol/L)	Standard deviation (pmol/L)	Repeatability coefficient of variation (%)
Canine	Low	798.7	98.65	12.35
	Mid	1,373.1	187.80	13.68
	High	2,822.8	303.95	10.77
Feline	Low	88.9	15.00	16.87
	Mid	160.3	15.71	9.80
	High	327.9	28.12	8.58

Table 2. Summary of results from canine and feline serum Catalyst proBNP Test precision study.

Interfering substances

The interfering substance study results are summarized in table 3. Hemolysis, lipemia, and icterus bias was minimal across all tested levels in both concentration pools and species.

Species	Interfering substance	Interfering level	Catalyst™ proBNP Test concentration	
			Low	High
Canine	Hemolysis	Control—nonspiked	322.4	1,277.0
		125 mg/dL	338.4	1,285.1
		250 mg/dL	324.1	1,289.4
		500 mg/dL	328.0	1,296.2
	Lipemia	Control—nonspiked	321.1	1,324.8
		125 mg/dL	310.2	1,296.9
		250 mg/dL	315.3	1,303.3
		500 mg/dL	307.9	1,335.6
	Icterus	Control—nonspiked	351.1	1,334.4
		1 mg/dL	326.1	1,347.1
		2 mg/dL	307.3	1,322.9
		4 mg/dL	322.4	1,335.7
Feline	Hemolysis	Control—nonspiked	90.7	323.8
		125 mg/dL	93.5	320.3
		250 mg/dL	94.5	316.7
		500 mg/dL	92.6	320.1
	Lipemia	Control—nonspiked	93.5	328.7
		125 mg/dL	95.3	332.0
		250 mg/dL	92.8	320.1
		500 mg/dL	95.4	324.7
	Icterus	Control—nonspiked	96.3	326.8
		1 mg/dL	98.9	330.6
		2 mg/dL	94.6	320.4
		4 mg/dL	95.0	331.0

Table 3. Summary of results from interfering substances study.

Influence of sample type

Catalyst proBNP Test results on paired serum and plasma samples showed no significant difference between matrices for either species (canine: $P = .445$, $n = 30$; feline: $P = .567$, $n = 22$), with interpretative zone (low/mid/high) concordance of 96.7% and 100%, respectively.

Conclusion

The Catalyst proBNP Test demonstrated low analytical bias, acceptable precision and strong correlation with the IDEXX Reference Laboratories Cardiopet™ proBNP Test. These performance features

indicate that the Catalyst proBNP Test is an accurate and reliable point-of-care assay for the quantification of NT-proBNP in dogs and cats.

When monitoring NT-proBNP concentrations it is recommended to use the same methodology in order to assess trends in results in the most accurate manner.

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