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Precise Diagnostics for Improved Care

Summary of Bionote Study

Validation of a Point-of-Care Quantitative Assay for Feline NT-proBNP

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Introduction

Cardiac biomarkers are substances that can be measured in the blood that can identify cardiac myocyte damage or stretch.

B-type natriuretic peptide (BNP) is a hormone that causes renal sodium and water loss and vasodilation. BNP is significantly upregulated in cardiac disease and failure in response to myocyte stress and stretch from increased volume and pressure.

BNP is secreted as a pro-hormone (proBNP) and is cleaved into the inactive N-terminal pro B-type natriuretic peptide (NT-proBNP) that can be measured in the blood. NT-proBNP is more stable and has a longer half life than BNP or proBNP and thus is the most ideal diagnostic analyte to assess the magnitude of myocardial wall stress or stretch (Fig 1).

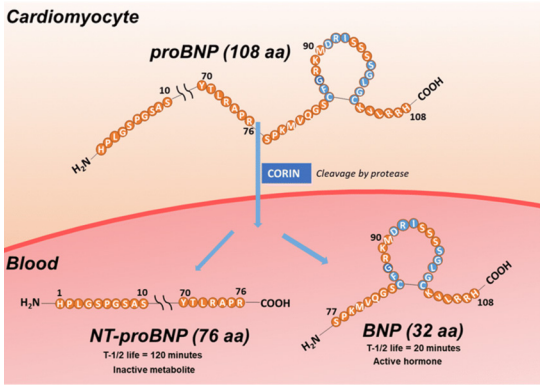
In veterinary patients, the use of quantitative NT-proBNP has been utilized with high sensitivity and specificity in the clinical setting to differentiate between cardiac and pulmonary disease for patients presenting with respiratory signs.

There is a point-of-care qualitative NT-proBNP colorimetric ELISA test available for feline patients, however, the likelihood of false positives is high, and it is recommended to follow up with an additional quantitative test.

Until recently, for quantitative analysis, samples required shipping to a commercial facility for testing and are subject to degradation from sample handling. Results may also take up to 72 hours to return.

A new NT-proBNP quantitative assay (Vcheck V200, Bionote Inc) is currently available. and the objective of this study was to independently validate this assay in accordance with the American Society for Veterinary Clinical Pathology (ASVCP) guidelines.

Figure 1



Materials & Methods

Serum samples from client-owned cats evaluated at the University of Illinois Veterinary Teaching Hospital were utilized. Serum samples were fresh or frozen for less than one week and allowed to thaw prior to analysis. Serum samples were pooled to obtain target concentrations of low (50 – 100 pmol/L), mid (101 – 300 pmol/L) and high (>301 pmol/L) and were stored at -80 °C until analysis.

Precision was determined for the low, mid, and high concentration pools within-day (short-term, 15 repetition) and within-week (long-term, 5 repetition each day for 5 days).

Linearity was performed by obtaining 10 NT-proBNP concentrations spanning the reportable range of the Vcheck analyzer and running the samples in triplicate.

Interference was determined for hemolysis, icterus, and lipemia using pooled serum with an NT-proBNP concentration of 394 pmol/L. Stock solutions for each interferent were prepared in accordance with the ASVCP guidelines. Samples for each level (1 – 4+) of interferent were performed in duplicate and interference indices were confirmed via a validated Olympus chemistry analyzer.

Bias was determined from stored (-80 °C) paired serum samples (n=49). One sample was submitted to IDEXX Laboratories for analysis using the CardioPet NT-proBNP assay and the other sample using the Vcheck analyzer. The duplicate samples were analyzed within the same week.

Preanalytical sample degradation was evaluated using fresh feline serum from 10 cats. Serum was stored at room temperature (20 °C or under refrigeration 4 °C) and NT-proBNP was evaluated using the Vcheck assay hourly for 6 hours and at 24 hours.

Statistical Analysis

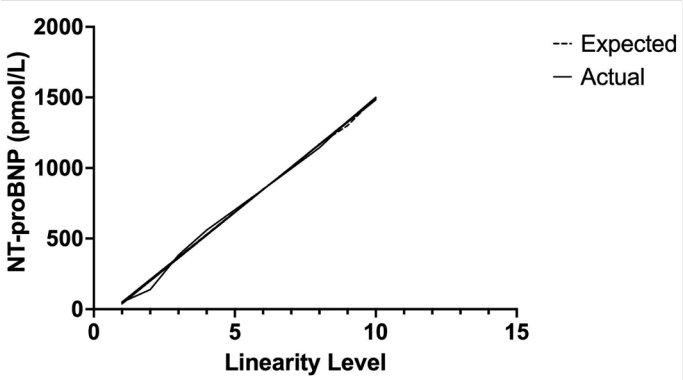
All statistical analysis was performed using commercially available software (Prism, MedCal). The coefficient of variation (CV) was determined from 15 within-day replicates and 25 within-week replicates for low, mid, and high concentration pools. Assays were compared using linear regression and Bland-Altman plots. Sample degradation was evaluated using a repeated measures ANOVA with a Dunnett's multiple comparison. A $P < 0.05$ was considered statistically significant.

Results

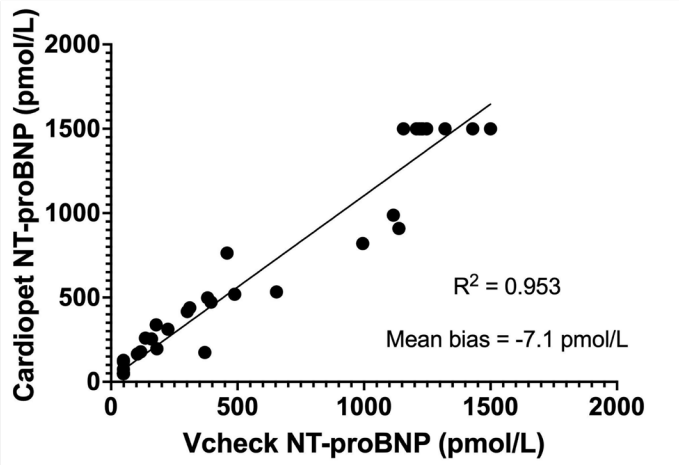
Analytical precision:

	Within-day		Within-week	
	NT-proBNP (pmol/L)	CV (%)	NT-proBNP (pmol/L)	CV (%)
Low	86.75	12.6	88.4	9.9
Mid	102	10.4	200.3	14.9
High	392.4	8.7	361	6.9

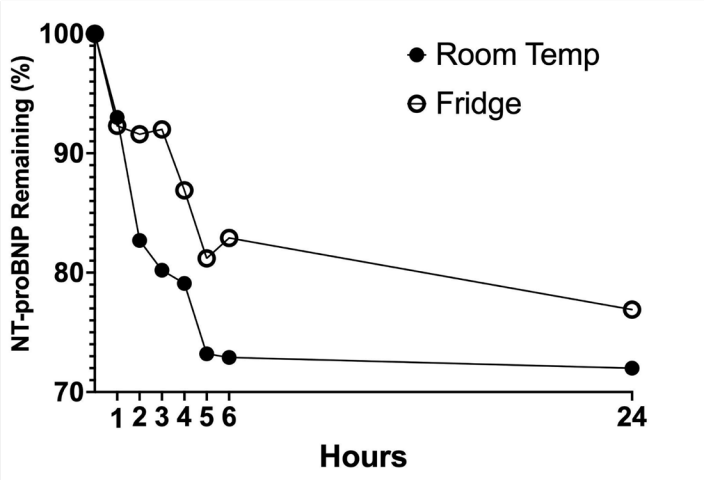
Linearity:



Analytical comparison:
Degradation:



Sample



Interference:
No significance interference was observed for hemolysis or lipemia. Values for all icteric samples were invalid.

Conclusions

The Vcheck 200 has acceptable precision, accuracy, and bias and can be a valuable point-of-care quantitative assay for measurement of feline serum NT-proBNP. Samples should ideally be processed within 2 hours of collection or refrigerated for up to 4 hours for best results.