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Cardiac troponin I concentrations in normal dogs and cats using a bedside analyzer

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Abstract *Objective:* To develop reference ranges for cardiac troponin I (cTnI) in normal dogs and cats using the Biosite Triage Meter®.
Background: Reference ranges for cTnI in dogs and cats have not yet been reported for this inexpensive bedside analyzer.
Methods: Purified free canine cTnI was diluted to 5 known concentrations to assess linearity and recovery. Interassay and intraassay precision were evaluated using 3 dilutions obtained from a dog with an elevated cTnI concentration. EDTA plasma was obtained from 55 normal dogs and 58 normal cats for analysis of cTnI.
Results: Measured values of purified cTnI closely matched calculated concentrations ($r^2 = 0.997$) and recovery ranged from 107—164%. Intraassay precision was 2.76 ± 1.20% and interassay precision was 8.50 ± 4.19%.

The dogs were 4.8 ± 3.1 years and 24.4 ± 11.2 kg (27 Mc, 19 Fs, 5 M, 4 F). The cats were 4.9 ± 2.8 years and 5.1 ± 1.19 kg (36 Mc, 22 Fs). The median and range (5th and 95th percentile) of cTnI for dogs were <0.05 ng/mL (<0.05—0.12). The median cTnI for cats was <0.05 ng/mL, as was the range, because only 3 cats (the upper 5% of the population) had detectable cTnI concentrations. The lower limit of detection for this assay is 0.05 ng/mL.
Conclusions: This study provides reference ranges for cTnI in dogs and cats using the Triage Meter®, an affordable bedside analyzer. The availability of reference ranges for this machine may increase clinical use and research of this marker in veterinary medicine.

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Introduction

Troponins are myofibrillar proteins that regulate the calcium-mediated interaction between actin and myosin in both cardiac and skeletal muscle. The troponin complex consists of troponin I which inhibits actin and myosin interaction, troponin C which binds calcium to relieve inhibition by troponin I, and troponin T which binds to tropomyosin.[1] Cardiac troponin I (cTnI) is the only one that is uniquely expressed in the myocardium.[2] Both cTnI and cardiac troponin T have been widely recognized as highly sensitive and specific blood markers for the noninvasive diagnosis of increased cardiomyocyte permeability, and blood concentrations appear to correlate with the extent of myocardial injury in people and animals.[3–11]

Free intramyocardial cTnI within the cytosolic pool only accounts for about 2–4% of the total cardiomyocyte troponin I in dogs and can be released without histological evidence of myocardial cell injury, accounting for a low background level.[3,6,7] Since the majority of cTnI is structurally bound within the cardiomyocyte, it is released into the circulation only after major injury with cell disruption and necrosis.[6,7,12] Troponins are detectable in the blood 5–7 h after cardiac injury, peak at 1–2 days and dissipate by 1–2 weeks in human beings with acute myocardial infarction and in dogs with experimental myocardial infarction.[1,13] The primary advantage of using cardiac troponins over more traditional markers of myocardial injury, such as CK-MB, is that they are more cardiospecific and the circulating concentration of cardiac troponin remains detectable for a longer period of time.[1,12,13]

Because of their high sensitivity and specificity, circulating cardiac troponins have become very important for the early detection of myocardial infarction in human beings.[1,14] Although myocardial infarction is uncommon in veterinary medicine, some dogs and cats with conditions involving myocardial damage, such as hypertrophic cardiomyopathy, unclassified cardiomyopathy, dilated cardiomyopathy, subaortic stenosis, mitral valve degeneration, gastric dilatation-volvulus, blunt thoracic trauma and babesiosis have been found to have elevated cTnI concentrations.[3–6,15,16]

The molecular structure of troponin proteins is highly conserved across species, and some current assays developed for their detection in humans have been used and validated in several other species.[2,17] Nucleotide sequencing of canine and feline cTnI has recently been published, demonstrating that canine cTnI showed 94% amino acid homology with human cTnI, feline cTnI showed 92% amino acid homology with human cTnI and canine cTnI showed 96% amino acid homology with feline cTnI.[18] Lack of standardization among different assays for cTnI is problematic for its clinical use because there are numerous cTnI assays in the market, and up to a 10-fold difference in values can occur largely due to differences in antibodies used in assay calibration.[19–21] Reference ranges have been established for dogs and cats using some machines, but because assays have not been standardized, they must be generated for each machine. The previously published papers establishing reference ranges in dogs and cats have used relatively expensive machines (all greater than 35,000 US dollars); the OPUS Immunoassay System,[a] Stratus CS stat flourometric analyzer,[b] and the Access AccuTnI.[c,3,6,12,17,22] The purpose of this study was to establish reference ranges in normal dogs and cats for cTnI using a less expensive bedside analyzer, the Biosite Triage Meter®[d] (analyzer 4000 US dollars, each test kit 25 US dollars). The establishment of reference ranges for this machine may allow rapid and relatively inexpensive cTnI analysis that is more readily available to veterinarians.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at the University of Florida. Purified free canine cTnI with purity > 98% as determined by SDS-PAGE[e] was reconstituted with a urea–tris buffer as recommended by the manufacturer. This mixture was diluted with canine plasma (that had undetectable cTnI using the Biosite Triage Meter®[d]) to concentrations of 12.50 ng/mL, 3.13 ng/mL, 0.78 ng/mL, 0.19 ng/mL and 0.04 ng/mL (detection limits for this analyzer are <0.05–30 ng/mL) to assess linearity and recovery. For each dilution, the recovery of cTnI is reported (recovery = {mean cTnI value measured by the analyzer/cTnI value expected based on the calculated dilution} × 100). Intraassay precision was assessed for the purified canine cTnI by running one test kit through the analyzer 3 times at each dilution and calculating the coefficient of variation (coefficient of variation = {standard deviation/mean of measured values} × 100). Purified feline

[a] Dade-Behring Diagnostics Inc, Westwood, MA, USA.
[b] Dade-Behring, Newark, DE, USA.
[c] Beckman Coulter Inc., Fullerton, CA, USA.
[d] Triage Meter®; Biosite Inc, San Diego, CA, USA.
[e] Advanced ImmunoChemical, Inc, Long Beach, CA, USA.