The IDEXX **Catalyst® CRP** Test for in-house measurement of C-reactive protein (CRP) concentration in serum from dogs

By Graham E. Bilbrough, MA, VetMB, CertVA, MRCVS; Paula W. Lampton, BA, PhD; Matt Wagner, BS; Sheila Corey, BS, MS; Dennis B. DeNicola, DVM, PhD, DACVP

Introduction

C-reactive protein (CRP) is an acute-phase protein that is measured to detect, characterize the severity and monitor systemic inflammation in dogs[1] and humans.[2] The rapid production and clearance of CRP makes it a very useful test, in combination with the complete blood count (CBC), to indicate the clinical situation of an animal at the time of sample collection,[3] particularly if measured in-house.

Interpreting canine CRP

Increasing concentrations of CRP support increasing severity of inflammation. CRP concentrations greater than 30.0 mg/L (SI units) indicate clinically significant systemic inflammation.

CRP concentrations will be significantly increased in less than 6 hours from the onset of significant inflammation and concentrations drop rapidly during resolution of the inflammatory process.

The IDEXX Catalyst® CRP Test comprises a new sandwich immunoassay with gold nanoparticles that is designed to measure canine CRP antigen (dynamic range 1–100 mg/L) in serum or lithium heparin plasma samples from dogs. The CRP slide may be added to a chemistry profile or run as a stand-alone test. It is designed to produce prompt and reliable test results in the veterinary clinic.

The objectives of this study were the following:

1. Method comparison of CRP concentrations determined by the Catalyst CRP Test (using either an IDEXX Catalyst One® or Catalyst Dx® chemistry analyzer) and CRP concentrations determined by a reference method* used by veterinary reference laboratories, including IDEXX Reference Laboratories.†
2. Understand the precision of the Catalyst CRP Test.
3. Understand the influence of common interfering substances on the reported results.

Method comparison

Materials and methods

Serum samples from 82 dogs, including a mixture of healthy animals and patients with significant inflammatory disease, were analyzed as follows:

1. Reference method: Gentian Canine CRP Reagent kit run on a clinical chemistry analyzer used in veterinary reference laboratories.† All samples were analyzed twice with the reference method and an average CRP concentration was calculated for use in the comparison.
2. Catalyst CRP Test: Each sample was analyzed once on two Catalyst One analyzers and two Catalyst Dx analyzers to give a maximum total of 4 comparisons per sample. The analyzers were used in a random order. For only two samples, there was insufficient volume for analysis on all four analyzers.

Both the reference method and Catalyst CRP assays were performed per the manufacturer's specifications. Analysis on all analyzers was completed within 4 hours.

Results from each Catalyst CRP Test run were compared to the average concentration from the reference method. Correlation plots were constructed with calculation of R-squared, slope, and mean bias. R-squared is a statistical technique that evaluates the relationship between two (r^2) or more (R^2) series of events, and the slope of this correlation directly speaks to the overall bias. In this context, an r^2 of one and a slope of one are a perfect correlation with zero bias.

Results

The results are summarized in table 1 and figure 1.

	Catalyst One	Catalyst Dx
Observations	162	161
r^2	0.96	0.97
Slope	0.98	0.98
Intercept (mg/L)	0.6	0.7
Mean bias (mg/L) in the range 1–100 mg/L	-0.2	0.0

Table 1. Summary of results from the comparison between the reference method and the Catalyst CRP Test

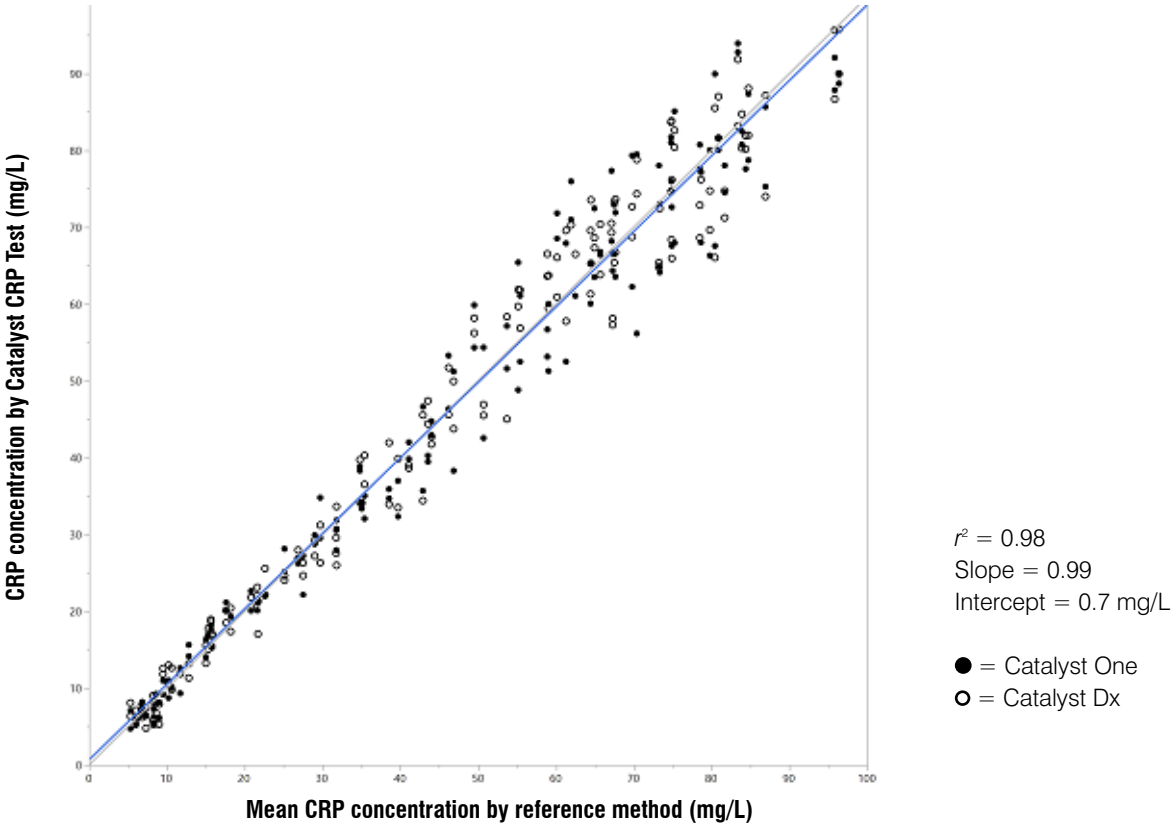

Figure 1: Correlation graph of pairwise comparisons (n = 323; 162 Catalyst One analyzers and 161 Catalyst Dx analyzers) for CRP concentrations in canine samples measured by the two assays. The line of best fit (linear regression) for the data is indicated in the correlation graph (blue line), with the slope and R-squared (r^2). X = Y is shown as the gray line.

Precision

Materials and methods

Precision was assessed using two levels of control fluid: control fluid 1 and control fluid 2. Two Catalyst Dx® and two Catalyst One® chemistry analyzers were evaluated with both fluids run on each analyzer eight times per day for 10 days. In total, each fluid was analyzed 80 times on each individual analyzer giving a total of 160 replicates on Catalyst One analyzers and 160 replicates on Catalyst Dx analyzers.

Observed percentage coefficient of variation (%CV) was calculated and compared to quality specifications detailed in the ASVCP guidelines for allowable total error:[4]

- Recommended desirable analytical CV for CRP (CV_{des}): 12.16%
- Recommended minimally acceptable analytical CV for CRP (CV_{min}): 18.24%

Results

Results are shown in table 2. The observed precision for both the Catalyst Dx and Catalyst One analyzers was far better than the ASVCP guidelines specifying desirable analytical CV.

	Control fluid	Mean concentration (mg/L)	%CV
Catalyst Dx	1	31	6.9
	2	76	7.6
Catalyst One	1	32	7.0
	2	79	6.8

Table 2. Summary of results from the precision study. %CV = percentage coefficient of variation.

Interfering substances study

Materials and methods

Interference caused by the presence of hemoglobin, lipids, or bilirubin was assessed per CLSI EP07-A2 method guidelines.[5] Canine serum samples, which were visibly clear of interferents, were collected and pooled. Canine red blood cell hemolysate,[‡] Intralipid®[§] and ditaurobilirubin[¶] were used for investigating potential impact by hemolysis, lipemia, and icterus, respectively. Aliquots of the pooled sample were then prepared and spiked with varying concentrations of the interfering substances (as shown in table 3). Each aliquot was then analyzed two times on a Catalyst Dx® analyzer and two times on a Catalyst One® analyzer.

Results

Results are shown in table 3. There was minimal, and clinically insignificant, interference by hemolysis, lipemia, and icterus.

Hemolysis		Lipemia		Icterus	
Hemoglobin concentration (mg/L)	Mean CRP concentration (mg/L)	Intralipid concentration (mg/L)	Mean CRP concentration (mg/L)	Ditaurobilirubin concentration (mg/L)	Mean CRP concentration (mg/L)
Not spiked	1.7	Not spiked	1.7	Not spiked	1.7
32	1.6	125	1.7	1.25	1.7
68	1.7	250	1.6	2.5	1.8
125	1.6	500	1.6	5	1.7
250	1.7	1000	1.7	10	1.8
375	1.7			15	1.8
500	1.6			20	1.7

Table 3. Summary of results from the interfering substances study (in Conventional Units). Each aliquot was measured, using the Catalyst CRP Test, twice on a Catalyst Dx analyzer and twice on a Catalyst One analyzer. The mean CRP concentration from the four replicates is shown.

Conclusion

The new Catalyst® CRP Test provides accurate and precise quantification of canine CRP in-house. There was minimal interference from hemolysis, lipemia, and icterus. It demonstrated excellent correlation ($r^2 = 0.98$) to the reference method and minimal bias (slope of 0.99).

*Reference method consisted of the Gentian Canine CRP Reagent kit (ref 1501; Gentian AS, Moss, Norway) performed on the Olympus AU400 Chemistry Analyzer (Beckman Coulter, Nyon, Switzerland).

†All IDEXX Reference Laboratories analyzing CRP, without referral to third-party reference laboratories, use the Gentian Canine CRP Reagent kit. That includes IDEXX Reference Laboratories in Japan and Europe.

‡Lysate from canine red blood cells washed in saline and lysed with Triton™ X-100 surfactant in water.

§Intralipid (Sigma, St. Louis, MO, USA), a phospholipid-stabilized soybean oil.

¶Bilirubin conjugate (Scripps Laboratories, San Diego, CA, USA; catalog number B0114), a synthesized ditaurobilirubin.

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Australia 1300 44 33 99

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