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ORIGINAL ARTICLE

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A new automated turbidimetric immunoassay for the measurement of canine C-reactive protein

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Background: In dogs, as in humans, C-reactive protein (CRP) is a major acute phase protein that is rapidly and prominently increased after exposure to inflammatory stimuli. CRP measurements are used in the diagnosis and monitoring of infectious and inflammatory diseases.

Objectives: The study aim was to develop and validate a turbidimetric immunoassay for the quantification of canine CRP (cCRP), using canine-specific reagents and standards.

Methods: A particle-enhanced turbidimetric immunoassay was developed. The assay was set up in a fully automated analyzer, and studies of imprecision, limits of linearity, limits of detection, prozone effects, and interferences were carried out. The new method was compared with 2 other commercially available automated immunoassays for cCRP: one turbidimetric immunoassay (Gentian CRP) and one point-of-care assay based on magnetic permeability (Life Assays CRP).

Results: The within-run and between-day imprecision were <1.7% and 4.2%, respectively. The assay quantified CRP proportionally in an analytic range up to 150 mg/L, with a prozone effect appearing at cCRP concentrations >320 mg/L. No interference from hemoglobin (20 g/L), triglycerides (10 g/L), or bilirubin (150 mg/L) was detected. Good agreement was observed between the results obtained with the new method and the Gentian cCRP turbidimetric immunoassay.

Conclusions: The new turbidimetric immunoassay (Turbovet canine CRP, Acuvet Biotech) is a rapid, robust, precise, and accurate method for the quantification of cCRP. The method can be easily set up in automated analyzers, providing a suitable tool for routine clinical use.

KEYWORDS
Acute phase proteins, C-reactive protein, Method comparison

INTRODUCTION

Some blood protein concentrations increase during inflammation as part of the host response to conditions that alter its homeostasis, known as the acute phase response (APR). These proteins are called acute phase proteins (APP), and their patterns of induction are species dependent. Many studies have shown that the dynamics of the APP during the APR reflect the underlying disease and can predict the disease outcome.[1] In dogs, as in humans, C-reactive protein (CRP) is a major acute phase protein. CRP concentration in the healthy state is very low, but increases rapidly and prominently after exposure to inflammatory stimuli, and can reach concentrations

several hundred times that of preinflammation concentrations.[2] Consequently, CRP has been established as a powerful biomarker for the detection and monitoring of inflammation and infection in human and veterinary medicine.[1] Canine CRP (cCRP) concentration increases in a variety of common infectious and inflammatory diseases.[1–3] Specifically, it has been reported to be a diagnostic test in dogs with steroid-responsive meningitis-arteritis (SRMA)[1,4], for early detection of postsurgical complications[5], and for monitoring disease progression and treatment efficiency in diseases such as pyometra[5,6], bacterial pneumonia[7], and the systemic inflammatory response syndrome (SIRS).[8] Interestingly, cCRP measurements can also be used to screen for occult disease in clinically healthy appearing dogs.[1,9]

Although CRP is a valuable biomarker, its inclusion in routine biochemical profiles of dogs has been limited for years, mainly due to the lack of adequate commercial analytic methods appropriate for clinical laboratories. To our knowledge, the first commercially available cCRP assay, at least in Europe, was an ELISA.[10] However, ELISAs are not optimal for routine analysis in clinical laboratories because they have relatively high variability, are time-consuming, and are unsuitable for single-sample processing. An in-house turbidimetric immunoassay for cCRP had been previously developed[11], but the assay was not available commercially. Automated turbidimetric immunoassays developed for human medicine with documented cross-reactivity with cCRP allowed the introduction of the CRP assay in routine biochemical panels in some veterinary laboratories, but these assays had serious limitations.[12,13] For instance, the use of human calibrators prohibited the direct extrapolation of cCRP concentrations, but rather required reporting results interms of human CRP equivalents. This hurdle can be overcome by the use of a purified cCRP as calibrator.[14] However, the necessity to use human CRP specific antibodies for detection requires validation of every new batch because reactivity with the homologous dog protein can vary from lot to lot affecting assay accuracy. A nephelometric immunoassay for cCRP has been available in Japan for a while.[3] More recently several other canine-specific automated immunoassays have become commercially available, including turbidimetric immunoassays, as well as different point-of-care (POC) systems.[15–17]

Here, we present a new automated particle-enhanced turbidimetric immunoassay for the measurement of cCRP based on antibodies against cCRP, and in combination with a canine CRP standard. The assay is now commercially available (Turbovet canine CRP, Acuvet Biotech, Zaragoza, Spain). As part of the validation study, the assay was compared with 2 other canine-specific commercial automated immunoassays: the Gentian method[15] and the Life Assays POC test (POCT).[16]

MATERIALS AND METHODS

Serum samples

Dog serum samples used in method validation were obtained for diagnostic purposes at the Hospital Clinic Veterinari (HCV), and submitted to the *Servei de Bioquímica Clínica Veterinària* (SBCV) (Universitat Autonòma de Barcelona, Spain) for routine diagnosis, or from healthy dogs or dogs with high CRP concentrations collected in another study (Number of approval PI56/15, Ethical committee, University of Zaragoza). For samples from animals belonging to private owners, the owners' consent was obtained. Sera were collected by centrifugation after clotting, and stored at −80°C for a maximum of 3 months until method comparison and validation studies were performed.

cCRP turbidimetric immunoassay

The cCRP concentration was determined using immunoturbidimetry, which measured increased absorbance at 600 nm after dilution of the serum sample with a reaction buffer including latex immunoparticles. Previously, anti-cCRP antibodies were isolated from anti cCRP-specific rabbit antiserum[18], followed by covalent fixation to carboxyl-modified polystyrene microparticles using the carbodiimide [1-ethyl-3-(3-dimethylamino-propyl) carbodiimide chloride] procedure, as previously described.[19] Assay calibration was performed with a multipoint calibration curve using a cCRP calibrator consisting of purified cCRP (150 mg/L) in a matrix based on a 0.1 M Tris Chloride, 0.15M NaCl, 3 mM $CaCl_2$ buffer at pH 7.4 and containing 10 mg/mL bovine serum albumin (BSA) and 0.09% sodium azide as a stabilizer. The development and establishment of the cCRP value in the calibration material by single radial immunodiffusion (SRID) using a cCRP standard serum has been previously described.[18]

Serial dilutions of the calibrator were prepared by manually diluting the cCRP calibrator in 0.05M Tris Chloride, 0.15M NaCl, 3.0 mM $CaCl_2$, 10% BSA, pH 7.4. The following cCRP concentrations were used to obtain the calibration curve: 150, 75, 37, 19, 9, and 0 mg/L (blank). Calibrators were processed in the same way as serum samples. Two control samples containing 20 and 90 mg/L of cCRP were also included every day during the validation study. The turbidimetric immunoassay was established on a fully automated analyzer Olympus AU400 (Hamburg, Germany). Final assay conditions used in the validation study are described in Table 1.

TABLE 1. Assay conditions for canine CRP analysis with the new turbidimetric immunoassay on an Olympus AU400 analyzer.

Sample volume (μL)	3
Buffer volume (μL)	230
Immunoparticles volume (μL)	70
Wavelength (nm)	600
Type of method	Fixed point
First reading (s)	216
Second reading (s)	450
Time of addition of immunoparticles (s)	180
Calibration method	Polygonal

Validation study

Precision was determined by analyzing 3 canine serum pools with low, medium, and high cCRP concentrations during 20 consecutive days, following the Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3.[20] Serum pools were analyzed in duplicate twice daily, with a minimum of 2 h between-runs. cCRP controls were included in each analytic run. The assay was calibrated once a week. Results from 80 runs per sample were analyzed by 2-way nested ANOVA. Within-run, between-run, between-day, and within-laboratory precision (total precision)[20] were obtained and expressed as CV.

Linearity was evaluated by diluting a canine serum sample with a high cCRP concentration (140 mg/L) (designated S1) with a saline solution (0.154 M NaCl) as the diluent. Serial dilutions with expected cCRP concentrations of 140, 80, 40, 20, 10, 5.0, and 2.0 mg/L were prepared, and analyzed in duplicate, in random order, and in a single analytical run. Resulting cCRP concentrations were plotted against expected values, and the best correlation between values was estimated using a regression line obtained by minimal square fitting. The linear range was estimated by visual assessment.[21]

The presence of a prozone effect was evaluated by analyzing serial dilutions of a canine serum sample (S2) containing 320 mg/L of cCRP using a saline solution (0.154 M NaCl) as the diluent. Expected cCRP concentrations were 320, 240, 160, 80, 40, 20, 10, and 5 mg/L. Additionally, a serum sample containing 460 mg/L of cCRP (S3) was spiked with a solution of purified cCRP (3,100 mg/L) to yield a cCRP concentration of 600 mg/L (S4). Samples S3 and S4 were assayed undiluted and prediluted at ratios of 1:5 and 1:10. cCRP concentrations of serum samples S2–S4 were also determined by SRID.

The limit of detection (LoD) was determined by measuring a blank sample, composed of 0.154 M NaCl and 50 g/L BSA, 30 times in one analytic run, and one sample with a low cCRP concentration (1.5 mg/L) 40 times in 5 analytic runs. The LoD was calculated with the following equation:

$$\text{LoD} = \mu_B + 1.645\sigma_B + 1.645\sigma_S$$

where μ_B is the mean concentration of the blank sample, σ_B is the SD of the blank cCRP sample concentrations, and σ_S is the SD of the low cCRP sample concentrations.[22]

The limit of quantitation (LoQ) was evaluated according to CLSI guidelines in EP17-A2.[22] A canine serum sample containing 11 mg/L of cCRP was serially diluted, and each dilution was analyzed 8 times in 5 analytic runs (a total of 40 replicates). The total error (T_e) was calculated for each of the dilutions using the following equation: T_e = bias + 2SD. Bias was the difference between the analytic value obtained for the sample (mean value) and the expected value (calculated from the value determined in the undiluted sample multiplied by the dilution factor), expressed as an absolute value if the difference was negative. The T_e was set at 29.6%.[15]

Interference from hemoglobin, bilirubin, and triglycerides was evaluated by spiking canine serum with increasing amounts of the interferents, as previously described.[23] Solutions containing 98 g/L of hemoglobin prepared from a hemolysate, 6 g/L of bilirubin in 0.1M NaOH (Sigma Aldrich, St. Louis, MO, USA), and 200 g/L of Intralipid (Fresenius Kabi AB, Uppsala, Sweden) for triglyceride concentrations were spiked into the canine serum samples. The samples were mixed to obtain final concentrations of 20 g/L hemoglobin, 0.150 g/L bilirubin, and 10 g/L triglycerides. Aliquots of the same serum samples were mixed in similar proportions with deionized water representing volume adjusted control sera. The spiked and the control sera were mixed in different proportions to obtain samples with varying concentrations of the interfering substances (Table 5). The interfering substance concentrations in the spiked samples were calculated taking into account the concentration of the concentrated solutions, the dilution factor used to prepare the samples, and the concentration of interferents in the control serum, which was set at zero. Samples were analyzed in duplicate and random order during a single run. Bias caused by the interferents (ie, differences between concentrations of cCRP in the sample containing the interfering substance and those of the control sample) were calculated and expressed as a percentage of the control serum. The presence of interference was defined as a bias ≥10%.

Method comparison

Method comparison was performed according to the ASVCP guidelines.[21] The new turbidimetric immunoassay was compared with 2 commercial assays: the Gentian cCRP assay (Gentian, Moss, Norway), which is a particle-enhanced turbidimetric immunoassay[15], and the Life Assays cCRP assay (Life Assays, Lund, Sweden), which is a magnetic permeability-based POCT immunoassay, with the reagent based on anti-cCRP magnetic nanoparticles and silica microparticles, both carrying covalently linked antibodies directed at cCRP.[16] Measurement procedures were compared by linear regression analysis according to the nonparametric Passing and Bablok method[24] and by analysis of differences according to the Bland and Altman method.[25]

A collection of 40 canine serum samples were analyzed with the 2 turbidimetric immunoassays using the same analyzer (Olympus AU400, Hamburg, Germany). All samples were measured in the same analytic run. Samples with cCRP values outside of the measurement range of the assays were excluded from the methods comparison study.

Samples were assayed randomly with the Life Assay method during 2 consecutive days because the Life Assays assay took 11 min per sample to determine cCRP concentrations.

RESULTS

cCRP turbidimetric immunoassay

Specific anti-cCRP antibodies raised in rabbits were covalently fixed to latex microparticles to develop a turbidimetric immunoassay with enough sensitivity to determine cCRP concentrations in canine serum samples. Figure 1 shows the dose–response curve obtained with purified cCRP under optimized analytic conditions (Table 1).

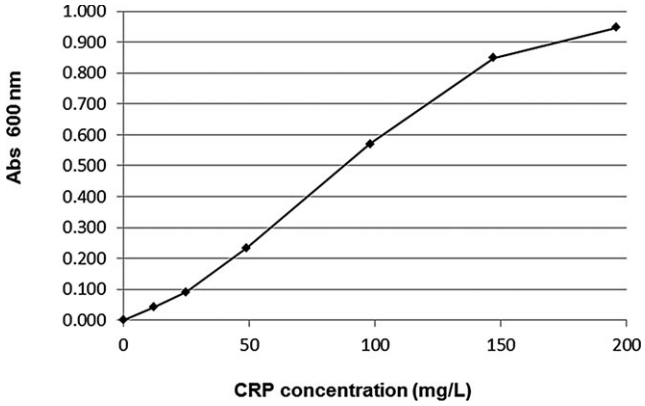

FIGURE 1. Dose–response curve of the turbidimetric immunoassay. Absorbance increases at 600 nm as a function of CRP concentration obtained with purified canine CRP with the assay conditions outlined in Table 1.

These conditions allowed a proportional cCRP measurement up to an achieved concentration of 150 mg/L, where a plateau was reached (Figure 1). The analytic range up to 150 mg/L was used in the subsequent validation studies.

Validation study

Results of the imprecision study are shown in Table 2. Within-run CV was <2% for all the samples, and total imprecision ranged from 1.52% (high cCRP samples) to 4.37% (low cCRP samples).

Linearity studies revealed that the assay measured proportionally in the analytic range up to 150 mg/L. A prozone effect was observed at concentrations >320 mg/L (Figure 2 and Table 3).

Results of the limit of quantification (LoQ) study are shown in Table 4. The assay LoD was 0.9 mg/L. The assay LoQ was established at 1.4 mg/L with a T_e of 21%, which is lower than the total maximum allowable error of 29.6% previously determined based on the biological variation of cCRP.[26]

The interference of hemoglobin, bilirubin, and triglycerides on cCRP measurements was evaluated (Table 5). No interference was detected for any of these substances at any of the doses evaluated (hemoglobin ≤20 g/L, triglycerides ≤10 g/L, bilirubin ≤0.150 g/L).

TABLE 2. Results of the imprecision study on a new immunoturbidimetric canine CRP (cCRP) assay using an Olympus AU400 chemistry analyzer. Three samples with low (sample 1), medium (sample 2), and high (sample 3) cCRP concentrations were analyzed daily in duplicate in 2 analytical runs for 20 days. Within-run, between-run, between-day, and within-laboratory (total) precision were obtained and expressed as CV as a percentage.

Sample	Mean cCRP (mg/L)	Within-run CV (%)	Between-run CV (%)	Between-day CV (%)	Within-laboratory CV (%)
1	11	1.19	0.94	4.09	4.37
2	36	1.60	1.02	2.84	3.42
3	135	1.22	0	1.07	1.52

The newly developed method was compared with 2 automated commercial immunoassays: the Gentian cCRP assay and the Life Assays cCRP method (Figure 3). A total of 33 samples were included in the comparison study with the Life Assays method after excluding those samples with cCRP values that fell outside of the assay analytical ranges. The cCRP concentrations determined in the canine serum samples with the 2 turbidimetric immunoassays (the new immunoassay and the Gentian assay) are compared in Figure 3.1.1. The regression equation, calculated according to the Passing and Bablok method, showed good agreement between the new immunoassay and the Gentian method:

New cCRP immunoassay (mg/L) = 1.048 (CI 1.019–1.077) × Gentian cCRP assay (mg/L) + 3.6 (CI 1.5–5.6)

Although the slope was statistically different from one and the x-intercept different from zero, the differences were minor and were considered clinically insignificant.

Figure 3.1.2 shows the Bland–Altman plot of the relative differences vs the mean values, including the 95% limits of agreement (−4.16 to 28.6%). The mean of the differences was 12.2% with a CI of 9.5–15.0%.

The cCRP values obtained with the new turbidimetric immunoassay also correlated with the values obtained with the Life Assays cCRP method, although a higher proportional error was detected compared with the Gentian cCRP assay (Figure 3.2.1). The cCRP values obtained with the Life Assays method were approximately 0.6 times lower than the values measured with the new turbidimetric immunoassay:

New cCRP immunoassay (mg/L) = 1.587 (CI 1.494–1.682) × cCRP Life Assays (mg/L) − 14.7 (CI −20.3 to −9.1).

Figure 3.2.2 shows the Bland–Altman plot of the relative differences vs the mean values for the new immunoassay and the Life Assays method, including the 95% limits of agreement (−107 to 135%). The mean of the differences was −13.74% with a CI of −35.3 to 7.9%.

DISCUSSION

Although CRP is recognized as a valuable biomarker for monitoring inflammation in the dog, its inclusion in routine biochemical profiles in dogs is not common, mainly due to the lack of available commercial methods appropriate for clinical laboratories. This hurdle is being overcome by the development of different automated immunoassays for cCRP analysis in canine serum samples. Turbidimetric immunoassays are very useful in clinical situations because of low variability, rapid results, and the possibility of automation with a continuous flow of analytic samples. The results of this study show that the new turbidimetric immunoassay developed by our group is a robust and accurate method that can be easily adapted to different clinical chemistry analyzers, thus providing a very useful tool for clinicians. This benefit is further validated by the good agreement of results

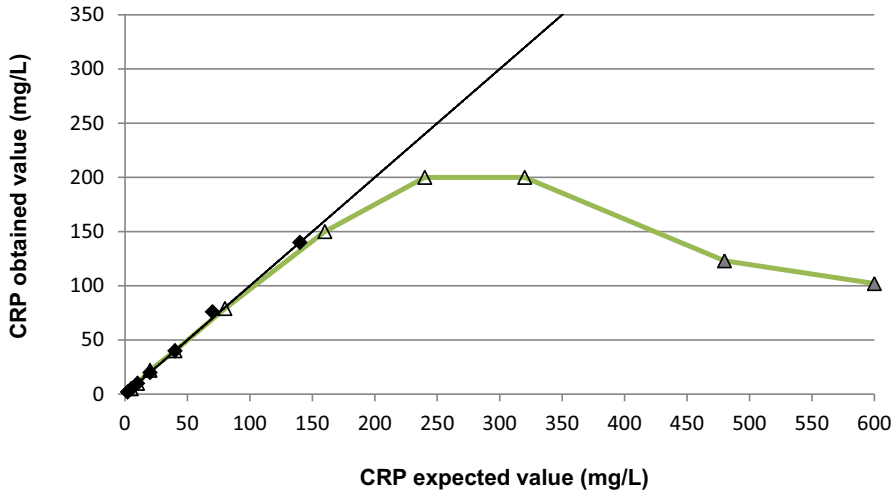

FIGURE 2. The linearity and prozone study. Two canine serum samples with high canine CRP concentrations (S1: 140 mg/L, black squares; S2: 320 mg/L, open triangles) were diluted and analyzed with the turbidimetric immunoassay. The obtained values are plotted against the expected values (theoretical values considering the dilution factor). To illustrate the prozone effect, the graph also includes canine CRP (cCRP) concentrations obtained with serum S3 (460 mg/L) and S3 spiked with cCRP (600 mg/L). Black line: Y=X.

TABLE 3. The prozone effect was evaluated by analyzing 2 serum samples (S2 and S3) containing 320 mg/mL and 460 mg/mL of canine CRP (cCRP), respectively, as determined by SRID. Additionally, the S3 serum sample was spiked with a solution of purified cCRP (3,100 mg/L) to yield a cCRP concentration of 600 mg/L (S4). Sample dilutions were carried out to yield a cCRP concentration falling into the measurement range of the assay, and the undiluted (S2–S4) and diluted samples were measured by the turbidimetric immunoassay. The measured value outlined in the table is the value obtained by the assay multiplied by the dilution factor.

Sample	cCRP (mg/L) in the sample	Dilution	Measured cCRP (mg/L)
S2	320	None	150
		1/4	320
S3	460	None	124
		1/5	480
S4	600	None	103
		1/10	572

TABLE 4. To determine the limit of quantification (LoQ) of the turbidimetric immunoassay, a canine serum sample with a low canine CRP (cCRP) concentration was diluted to obtain samples with the expected values outlined in the first column (cCRP expected value). The samples were measured 40 times in 5 runs, and the mean, the SD, the bias with respect to the expected value, and the total error (T_e) were calculated. The LoQ of the assay was found to be 1.4 mg/L with a T_e of 21%.

cCRP expected value (mg/L)	Mean	SD	Bias	T_e (%)
5.5	5.4	0.24	−0.1	11
2.7	2.7	0.18	0	13
1.4	1.4	0.15	0	21

obtained at different laboratories across Europe using the assay with different clinical chemistry analyzers.[27]

The use of specific reagents is fundamental for the development of robust and accurate species-specific turbidimetric immunoassays. Our antibodies are very specific because the antiserum is immunoadsorbed with canine serum deprived of cCRP.[18] Accordingly, the dose–response curve obtained with canine serum was similar to that obtained with purified cCRP, and no matrix effect was revealed in the linearity under dilution study.

The imprecision of the new cCRP turbidimetric immunoassay was very low: within-run CV was lower than 2% for all the samples tested, and total imprecision ranged from 1.52 (high cCRP sample) to 4.37% (low cCRP sample). These values are well within the maximum allowable imprecision of 12% previously suggested for cCRP determination, due to the biologic variability determined in healthy dogs.[15–26] The imprecision of our assay is similar to that reported for the Gentian cCRP assay[15], and lower than that reported for the Randox method (intra-assay CV of 1–10% and inter-assay CV of 6–18%)[12], the Life Assay (10% CV for samples with cCRP concentrations above 50 mg/mL)[17], and other POC systems such as the Teco Medical system (CV 19% for samples with CPR <20 mg/L), or the Eurolyser system (CV 14% for samples with cCRP concentrations of 50–100 mg/L).[17] Higher intra- and inter-assay CVs have also been reported for an ELISA method (in the range of 6.9–10.1% and 7.5–29.0%, respectively).[10]

The turbidimetric immunoassay was optimized to give a dynamic range of up to 150 mg/L, similar to the Randox method for cCRP. The linearity in this range is excellent and allows the accurate measurement of the large majority of clinical samples including those with CRP values in the normal range and most pathologic samples. Although a prozone effect was observed at cCRP concentrations higher than 320 mg/L, even samples as high as 600 mg/L gave results >100 mg/L, 5 times higher than the reference value of

TABLE 5. The interference study was performed by measuring canine CRP (cCRP) concentrations in serum samples supplemented with increasing amounts of hemoglobin, bilirubin, and triglycerides. Interference was defined as the bias between cCRP concentrations measured in the supplemented sample compared with that measured in the unsupplemented control samples and expressed as a percentage of the control.

Samples supplemented with hemoglobin								
Sample ID	H0	H1	H2	H3	H4	H5	H6	H7
Hemoglobin (g/L)	0	1	2	4	8	12	16	20
cCRP (mg/L)	31	31	31	31	31	31	32	31
Interference (%)	–	0	0	0	0	0	3.2	0
Samples supplemented with bilirubin								
Sample ID	B0	B1	B2	B3	B4	B5	B6	B7
Bilirubin (g/L)	0	0.008	0.015	0.030	0.060	0.090	0.120	0.150
cCRP (mg/L)	36	37	38	37	36	36	36	37
Interference (%)	–	2.8	5.6	2.8	0	0	0	2.8
Samples supplemented with triglycerides								
Sample ID	T0	T1	T2	T3	T4	T5	T6	T7
Triglycerides (g/L)	0	0.5	1	2	4	6	8	10
cCRP (mg/L)	36	36	37	36	37	36	36	36
Interference (%)	–	0	2.8	0	2.8	0	0	0

20 mg/L that was established for systemic inflammation[28], and corresponded to a pathologic sample. Due to the lack of analytic method harmonization in the literature, the maximum cCRP serum concentration in sick dogs is not easily determined. The highest cCRP concentration measured in dogs with systemic inflammation of different origins using the Randox method and a cCRP calibrator[28] was around 400 mg/L, and more than 90% of the samples measured in this study were <300 mg/L. Therefore, the method developed in the present work is adequate for the measurement of routine clinical samples, allowing the correct discrimination between healthy animals and animals with inflammatory disorders. On the other hand, according to Figure 2 and Table 3, to avoid the prozone effect that may occur with very high cCRP concentrations, samples with cCRP concentration >120 mg/L (security range up to 450 mg/L) or 100 mg/L (security range up to 600 mg/L) can be diluted by 1/6 to fall into the linear range, and then be reanalyzed. The security range could be increased (ie, the prozone effect would appear at a higher cCRP concentration) if the sample volume is reduced (eg, 2 μL instead of 3 μL). Our validation was performed with 3 μL of the samples because it is a volume adequate for all analyzers and allows an accurate measurement of the samples with low cCRP concentrations.

In the analytic conditions selected, the LoQ of the assay was 1.4 mg/L. This value is lower than those reported for other automated immunoassays such as the Gentian cCRP assay (7 mg/L)[15], the Life Assay (7 mg/L), and other POC systems evaluated in the literature (10–20 mg/L).[17] Although these comparisons have to be taken with caution, due to the lack of harmonization among the different commercially available assays, the method described here allows the quantification of samples from healthy dogs with high accuracy. It can be argued that low cCRP values are not relevant for clinical purposes. However having a valid analytic concentration value for samples with low cCRP concentration is advantageous when statistical analyses are performed in a clinical study and would be of interest in the assessment of minimal inflammation, and to compare baseline levels in different populations or breeds.

The lack of interference by hemoglobin or triglycerides in our analytic results is also essential, as hemolytic or lipemic serum samples are common in sick dogs. The presence of hemoglobin in samples can increase concentrations measured by ELISA[29] and decrease cCRP concentrations measured by turbidimetry with nonparticle-enhanced methods.[11] Substantial background absorption caused by hemoglobin has been suggested to be the cause of the marked decreases in cCRP concentration observed with one turbidimetric immunoassay (up to 90% with 80 mg/dL hemoglobin).[2,11] This artifact is eliminated with our particle-enhanced method because it measures at 600 nm where the background absorption of hemoglobin is irrelevant considering the amount of serum used in the reaction. In addition, the background is corrected by performing the first measure after the addition of sample, buffer, and reagent. Hyperbilirubinemia also does not affect the cCRP concentration measured by our assay, in contrast to the cCRP results obtained by ELISA.[29] The maximum concentrations of the substances tested in our study are similar or higher than those evaluated in other cCRP method studies.[15,29]

When the analytical results, obtained with the Gentian assay and the new turbidimetric immunoassay were compared, only small proportional and constant errors were detected. The agreement between the new turbidimetric immunoassay and the Gentian method can be considered very good, taking into account that the assays use different unrelated standards for calibration. Furthermore, the analysis of differences showed limits of agreement that can be considered acceptable according to the total allowable error of 29.6% previously determined for cCRP measurements and based on

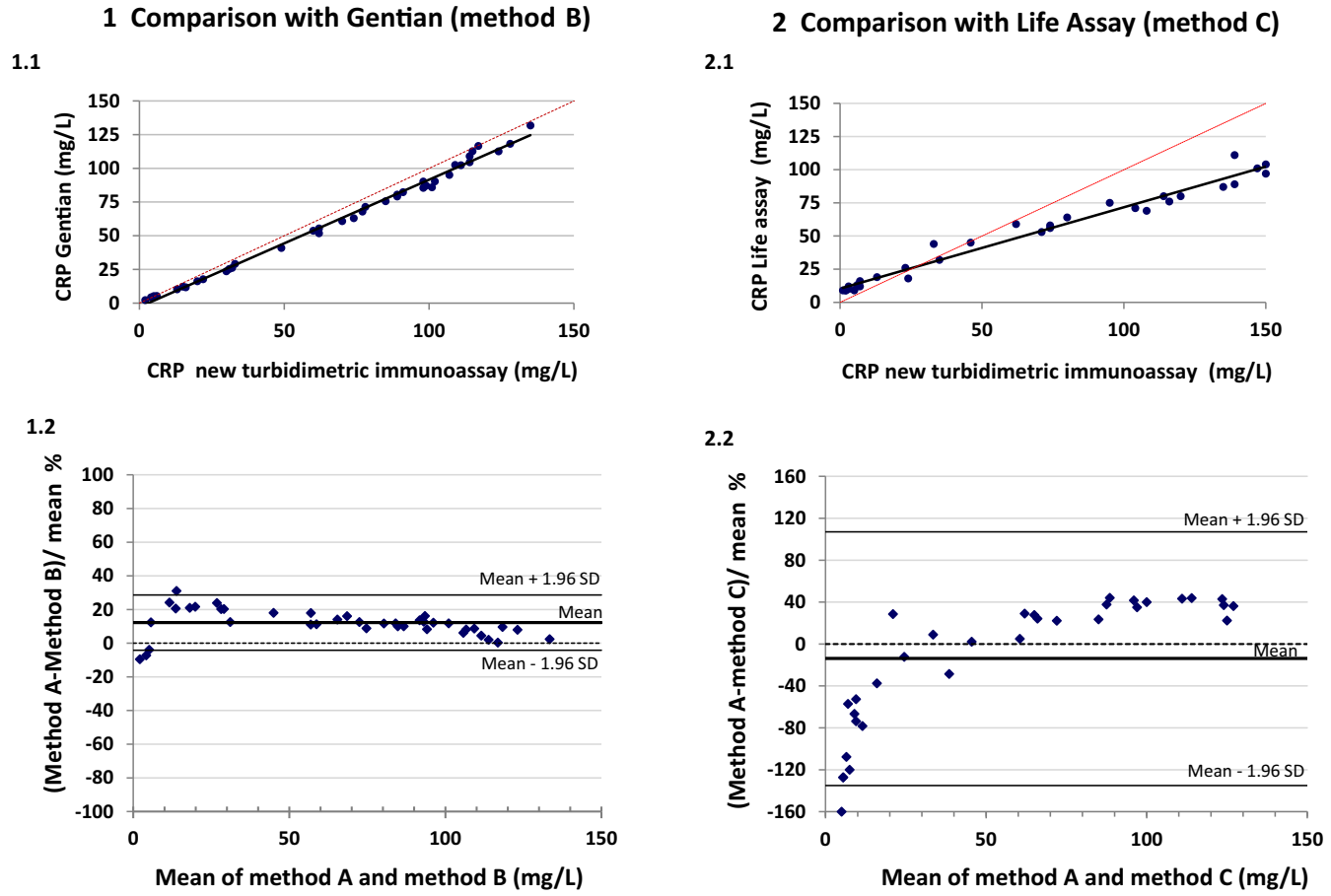

FIGURE 3. Method comparison studies. (1) Comparison of the new turbidimetric immunoassay and the Gentian Assay. 1.1) Linear regression analysis. Values obtained with the 2 assays were plotted and adjusted to a line by linear regression (black line). The dotted line shows Y=X. 1.2) The Bland–Altman difference Plot. *Y*-axis: canine CRP (cCRP) value obtained with the new turbidimetric immunoassay (method A) minus cCRP value obtained with the Gentian method (method B), expressed as a percentage of the mean. *X*-axis: mean of the cCRP concentrations obtained with the 2 methods. The mean of the differences (line marked as mean) and the 95% limits of agreement (mean ± 1.96 SD) are included in the graph. 2) Comparison of the new turbidimetric immunoassay and the Life Assays method. 2.1) linear regression analysis. Values obtained with the 2 assays were plotted and adjusted to a line by linear regression (black line). The dotted line shows Y=X. 2.2) The Bland–Altman difference Plot. *Y*-axis: cCRP value obtained with the new turbidimetric immunoassay (method A) minus cCRP value obtained with the Life Assays method (method C), expressed as a percentage of the mean. *X*-axis: mean of the cCRP concentrations obtained with the 2 methods. The mean of the differences and the 95% limits of agreement (mean ± 1.96 SD) are included in the graph.

biologic variability.[15,26] The values obtained with the Life Assays method were correlated with the values obtained with the new turbidimetric immunoassay, but an important proportional error was observed. These discrepancies are probably mainly due to the different standards used for calibration and point out the necessity of using appropriate reference materials for assay calibration to harmonize the results among laboratories. Our group has addressed this question and studies to develop reference material for harmonization of cCRP assays have been carried out.[27]

In conclusion, our new particle-enhanced turbidimetric immunoassay is a robust, accurate, and precise method, which represents a good alternative for the routine analysis of cCRP in laboratories where a clinical chemistry analyzer is available. With the assay conditions described in this study, a prozone effect could appear with extremely high cCRP concentrations; however, it should not interfere with the clinical decision-making about cCRP sample concentrations. If an accurate measure of very high cCRP samples is required, the prozone effect can be avoided by diluting and reanalyzing samples giving cCRP values >100 mg/L.

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DISCLOSURE

The assay described here is currently commercially available at the company Acuvet Biotech. This study was performed independently

before the company was founded. MP and LS are currently working for Acuvet Biotech. MP is among the founders of Acuvet Biotech.

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