VALIDATION OF A NEW CANINE-SPECIFIC DRY CHEMISTRY ASSAY FOR THE QUANTIFICATION OF CANINE C-REACTIVE PROTEIN

Alberto Muñoz Prieto[1]; Silvia Martínez Subiela[1]; Asta Tvarijonaviciute[2]; Damián Escribano Tortosa[1]; José Cerón Madrigal[1]

[1]*Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), Veterinary School, Campus of Excellence Mare Nostrum, University of Murcia, 30100 Murcia, Spain*

[2]Departament de Medicina i Cirugia Animals, Universitat Autónoma de Barcelona, 08193 Barcelona, Spain.

Background

C-reactive protein (CRP) is a major positive acute phase protein (APP) in dogs, produced mainly in the liver in response to increased concentrations of pro-inflammatory cytokines[1] in a wide variety of diseases in dogs, such as: infectious and immune-mediated diseases or neoplasia[1]. Therefore, CRP measurement is one of the most sensitive tests to detect inflammation in this species that can be done in clinical practice[2].

Objective

The objective of the current study was to validate the use of a new canine-specific dry chemistry assay for in-house CRP measurement (FUJI DRI CHEM SLIDE vc-CRP-P, FUJIFILM®) (Figure 1). For this purpose, an analytical validation was conducted, as well as the evaluation of the assay to detect different serum CRP concentrations between healthy and dogs with inflammation.

Material and methods

-Samples. 15 canine serum samples were selected to create three pools (5 serum samples each one):

- 1 pool with high CRP concentration (100 mg/L)
- 1 pool with medium CRP concentration (50 mg/L)
- 1 pool with low CRP concentration (10 mg/L)

Figure 1. Fuji Dri-Chem NX 500®

-Analytical validation. The following parameters were determined:

- Precision:
  - Intra-assay precision was determined by measuring each of the pools 5 times in the same analytical series (Table 1).
  - Inter-assay precision was done measuring each pool in 5 different days (Table 1).
- Accuracy was indirectly assessed by linearity under dilution (Figure 2).
- Sensitivity:
  - Limit of detection (LD) was determined from 12 replicate measurements of zero standard (assay diluent) as mean value +2 SD (Table 2).
  - Lower limit of quantification (LLOQ) was the lowest CRP concentration that could be measured which the coefficient of variation (CV) of 5 repeated determinations below 20% (Table 2).

-Clinical validation. 36 canine serum samples (20 with inflammatory diseases and 16 healthy) was selected from our database (Figure 3).

Results

* Analytical validation

Table 1. Coefficient of variation (%) for the intra and inter-assay.

Pool	Intra-assay	Inter-assay
High	2.01%	9.45%
Medium	2.64%	3.55%
Low	7.9%	14.4%

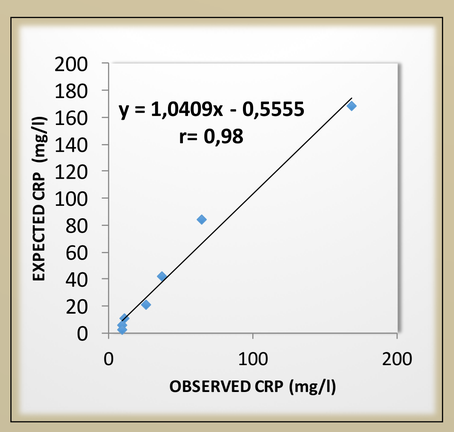

Figure 2. Linearity under dilution in high CRP concentration pool of canine serum.

Table 2. Limit of detection (LD) and lower limit of quantification (LLOQ).

Limit	CRP (mg/L)
LD	3
LLOQ	10.4

* Clinical validation

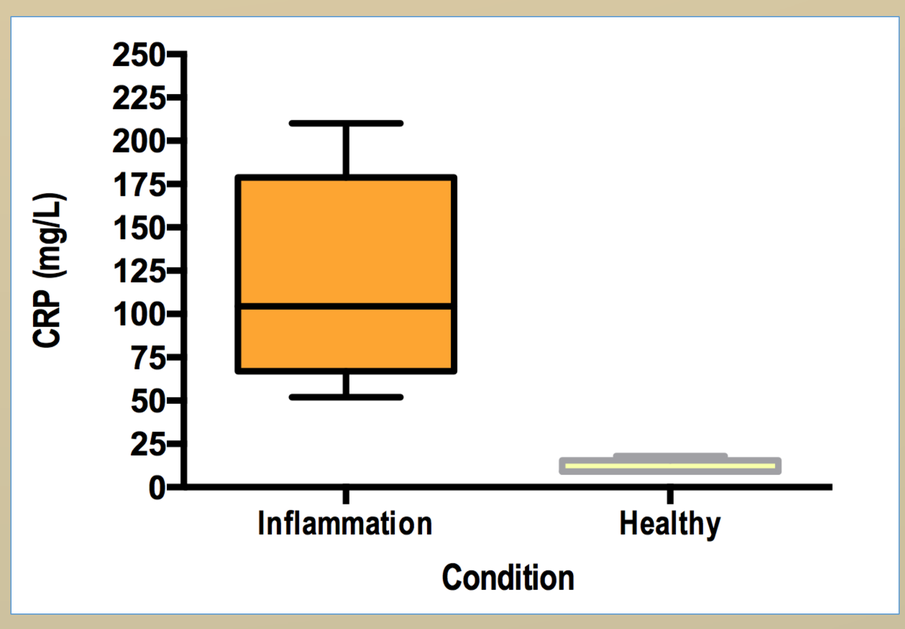

Figure 3. Comparative C-reactive protein (CRP) concentrations in dogs with different conditions (inflammation and healthy).

Discussion and conclusions

The precision study showed that the CVs were acceptable for the pools with high and medium CRP concentrations. Although high CVs were observed with low CRP concentrations, it has been observed that the maximum value of CRP to consider not inflammation can be established at 12 mg/L,[3] well above of the LLOQ detected in this assay and, therefore, would not have a major implication in the clinical decision in order to rule in or rule out active inflammation or sepsis.

Analytical results provided in this study indicate that the method can measure high and medium CRP concentrations with adequate precision and accuracy. In addition, from the clinical point of view, the assay was able to detect changes in CRP levels in dogs with inflammatory diseases compared with healthy dogs.

References

1. Cerón, J. J., P. D. Eckersall, S. Martínez-Subiela. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet. Clin. Pathol. 2005;34(2): 85-99.
2. Cerón JJ, Martínez-Subiela S, Ohno K, Caldin M. A seven-point plan for acute phase protein interpretation in companion animals. Vet J 2008;177:6-7.
3. Yamamoto S, Shida T, Okimura T, et al. Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test. Vet Q 1994;16:74-77.