**EFFECTS OF HAEMOLYSIS, LIPAEMIA AND BILIRUBINAEMIA IN A TR-IFMA FOR CANINE CRP**

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**Introduction**

C-reactive protein (CRP) is one of the major acute phase proteins in the dog. Currently a commercial ELISA is the method of choice for measuring the protein in canine specimens, however this assay showed interferences when using haemolysed, lipaemic or hiperbilirubinaemic samples. Recently, a new method for measuring canine CRP has been developed (Parra et al. 2005), but not assessed for haemolysis, lipaemia or bilirubinaemia. The aim of the present study was to evaluate the effect of increasing concentrations of haemoglobin, lipids and bilirubin in CRP measurements by this new technique.

**Materials and Methods**

The effects of these interfering substances in canine CRP determination were assessed following previously reported protocols (Jacobs et al. 1992; Lucena et al. 1998) except for the haemolysis trial. In this case, haemolysis was produced by freezing red cells at –20°C in order to not incorporate distilled water to the assay. The haemolysate was added to pooled serum at final concentrations of 0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 g/dL. A commercial emulsion of triglycerides (Lipofundina MCT/LCT 20%, Braun Medical, S.A.; Barcelona) was added to homologous pooled sera at 0.0, 31.2, 62.5, 125, 250 500 and 1000 mg/dL. Bilirubin (Sigma-Aldrich, Spain) was initially dissolved in dimethyl sulfoxide (DMSO) and then added to pooled sera at 0, 3.75, 7.5, 15, 30 and 60 mg/dL. DELFIA Diluent I was used to prepare haemoglobin and lipid series and DMSO to the bilirubin series. Samples were analyzed by a Wilcoxon Signed-Rank test (SPSS software, SPSS Inc, Chicago, Ill).

**Results**

The effect of the interfering substances can be appreciated in the corresponding interferographs (Figures 1-3) where data points represent the mean of duplicate determinations, X axes increasing concentrations of haemoglobin, lipid or bilirubin and Y axes percentage change of C-reactive protein for a given concentration of the added substances.

Addition of fresh haemolysate, tryglicerides or bilirubin to serum samples did not affect CRP concentrations ($P \geq 0.18$).
Discussion
The stable europium-chelate used as label in the assay enables the measurement of the time-resolved fluorescence that is virtually free from the background signal derived from the sample components and plastics. Furthermore, the dilution factor used in our study is higher than that used for the commercial assays and this is likely to have a positive contribution in reducing any assay interference from blood components. The TR-IFMA could be an alternative to the traditional tests for canine CRP, with the advantage of not being affected by haemolysis, lipaemia and bilirubinaemia.

References