**ANALYTICAL VALIDATION OF CANINE KIDNEY INJURY MOLECULE-1 (KIM-1) IMMUNOASSAY IN URINE SAMPLES**

**ANALITIČKA VALIDACIJA IMUNOENZIMSKOG TESTA ZA ODREĐIVANJE KONCENTRACIJE MOLEKULE BUBREŽNE OZLJEDE 1 (KIM-1) U URINU PASA**

**Josipa Kuleš1, Petra Nižić2, Blanka Beer Ljubić2, Renata Barić Rafaj3, Vladimir Mrljak1,2**

*1 ERA Chair team (VetMedZg), Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

*2 Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

*3 Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Croatia*

The kidney injury molecule-1 (KIM-1) is a novel biomarker for prediction, diagnosis and prognosis of acute kidney injury. The aim of this study was to evaluate commercially available canine-specific KIM-1 ELISA (Immunology Consultants Laboratory, Inc., Portland, USA) for use in canine urine samples.

For assay precision, two pools of urines with different concentrations of analyte that corresponded to low and high ranges of the assay were prepared from samples obtained from dogs. Intra-assay coefficient of variation (CV) was calculated, after analysis of the high and low pool, six times in a single assay run. Inter-assay CV was determined by analysing the same sample in five separate runs carried out on different days. Two samples with different amount of analyte were mixed in different ratios to perform a spiking recovery test. Test recovery, expressed as a percentage, was calculated for each dilution for comparison of expected versus measured concentrations. To investigate the effects of haemolysis, lipiduria and bilirubinuria, canine pools were mixed with different concentrations of haemoglobin, lipid or bilirubin solution.

Intra- and inter-assay coefficients of variation (CV) for assay were both below 10%, *i.e.* for low range pool CV was 9,98% (mean ± SD: 12,03 ± 1,2 ng/ml); for high range pool 8,83% (62,57 ± 5,53 ng/ml); and for inter-assay 8,39% (13,81 ± 1,16 ng/ml). The recovery between observed and expected concentrations ranged from 94 – 108%, with a mean of 101%. The different degrees of bilirubinuria tested in this study did not affect the measured concentrations of KIM-1 in the canine urine samples, while significant increase in KIM-1 concentrations was found in the presence of severe haemolysis (> 5 g/l) and also in marked lipiduria (triglycerides > 5 g/l).

This method exhibited acceptable analytical characteristics, allowing its use in the laboratory with an adequate precision and recovery, with exception of highly haemolysed and/or lipuric urine samples.