Validation of a new canine species-specific C-reactive protein assay on the Pentra 400

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Introduction & Aim

Rapid immunoturbidimetric detection of canine C-reactive protein (CRP) replaced CRP detection with ELISA-methodology but was hampered by the use of a human CRP test and labour intensive by preparation of pool plasma as canine controls. Costly purified canine CRP was further needed to establish a species-specific calibration curve.
Now a new canine-specific immunoturbidimetric CRP assay is available which provides canine-specific reagents, controls and calibrators.

Aim of the study was the validation of this new assay (by **Gentian AS,** Moss, Norway) against the previously validated immuno-turbidimetric assay from **Randox** Laboratories Ltd (Crumlin, UK) [1] on the wet chemistry analyzer **Pentra 400** (ABX horiba diagnostics).

Material and Methods

Inaccuracy: Linearity under dilution
- Patient serum 281.3 mg/l CRP diluted with NaCl to 7 different levels, each level was measured in tripclicates

Intra- and inter-assay precision:
- 4 different levels of patient sera:
  A: 7.15 mg/l, B: 58.36 mg/l, C: 103.86 mg/l, D: 272.07 mg/l CRP
- measured daily, twice in the morning and twice in the evening for 5 consecutive days

Interference:
- aliquots of canine patient serum of 35,5 mg/L CRP spiked with 800 mg/l bilirubine, 5 g/l hemoglobin, or 10 g/l intralipid were analyzed in triplicates and compared to aliquots spiked with either NaOH, NaCl or A. dest.

Evaluation of possible prozone effekt:
- Three different CRP samples were used to assess hook-effect: 455 mg/l, 676 mg/l, 890 mg/l

Method comparison:
- 279 sera of healthy and diseased dogs
- Comparison with immunoturbidimetric assay from Randox Laboratories Ltd, Crumlin, UK [1]

Statistical analysis

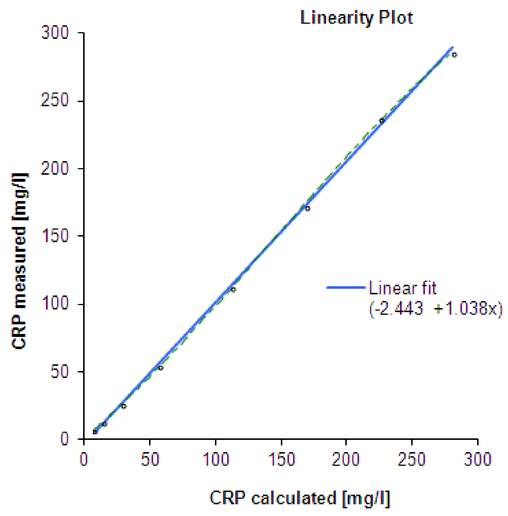
- Mean and standard deviation (SD), coefficient of variation (CV)
- Spearman´s rank correlation coefficient
- Bland-Altman analysis

- Quality specifications were set according to the ASVCP TE_a guidelines [2]:
  - TE_a optimal/ desired: 14.79%/ 29.58%
  - CV optimal/ desired: 6.06%/ 12.16%
  - bias optimal/ desired: 4.76%/ 9.52 %

Results

Inaccuracy:

Linearity under dilution was present up to 280 mg/l.



Precision:

Optimal quality specifications could be met. CV of the lowest CRP level was lower than the desired CV% outlined by the ASVCP.

	Within-run [mg/l]			Between-run [mg/l]		
Level	**Mean**	**SD**	**CV%**	**Mean**	**SD**	**CV%**
A	7.15	0.87	12.12	7.15	0.56	7.84
B	58.36	1.08	1.85	58.36	1.25	2.14
C	103.86	0.88	0.85	103.86	0.91	0.88
D	272.07	1.85	0.68	272.07	4.98	1.83

Interference:

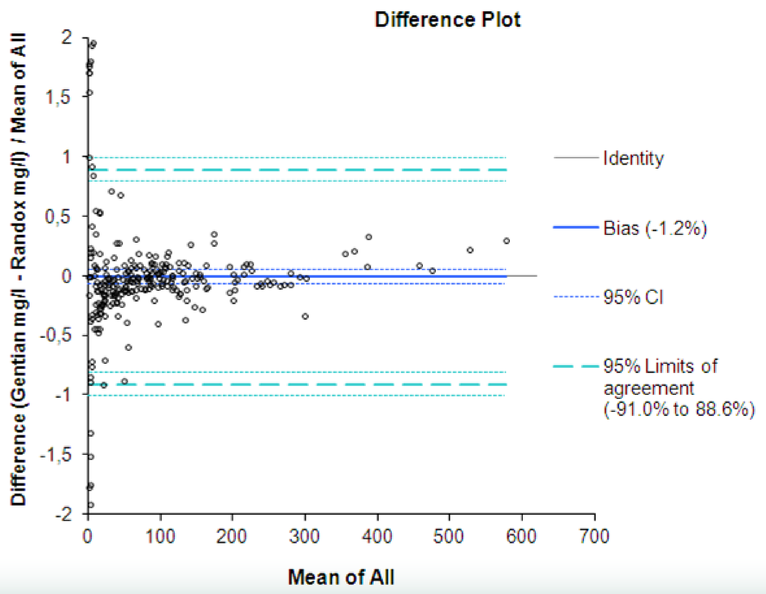
Mean absolute bias calculated was 0.57 mg/l, 0.13 mg/l, and 1.63 mg/l for the interferent bilirubine, hemoglobin and intralipid.
At a clinical decision level of i.e. 30 mg/l CRP, the optimal TEa would be 4.44mg/l in absolute numbers. Observed systematic error is lower than the absolute total allowable error in all interfering substances.

Evaluation of possible prozone effect:

Recovery rates for the detection of 455 mg/l CRP were 130% for 676 mg/l 121% and for 890 mg/l 28%. No prozone effect was present up to a concentration of 676 mg/l CRP.

Method Comparison:

- Spearman´s rank correlation coefficient (*r*), *n*= 263, 12 excluded due to missing data, *r*= .99
- Bland-Altman analysis, *n*= 263, 12 excluded due to missing data, bias -1.2%



Conclusions

The new Gentian canine CRP assay precisely and accurately detects canine CRP in comparison to the previously used immunoturbidimetric method. Advantage is the availability of a whole test kit including species-specific reagents, calibrators, and controls.

References

[1] Klenner S, Bauer N, Moritz A. Evaluation of three automated human immunoturbidimetric assays for the detection of C-reactive protein in dogs. J Vet Diagn Invest. 2010 Jul;22(4):544-52.
[2] K.E. Harr, B. Flatland, M. Nabity, K.Freeman, ASVCP Allowable Total Error Recommendations for Biochemistry, http://www.asvcp.org/pubs/qas/index.cfm, assessed 24th of September, 2013

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The sole responsibility for the study's content lies with the authors.