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Validation of a feline-specific immunoassay for the quantification of Serum Amyloid A (sAA) in cats

Serum Amyloid A (SAA) is a major positive acute-phase protein (APP) in cats.[1] Increased SAA concentrations have been observed in a wide variety of diseases[2] in cats such as: neoplasia[3], enteritis and pancreatitis[4], chronic kidney disease[5], pyometra[6], feline infectious peritonitis[7] or panleukopenia[8]. Overall, currently, SAA measurement is one of the most sensitive tests to detect inflammation and it is very useful in clinical practice. The use of SAA measurement in routine can be favoured by the existence of in-house methods that ensure the prompt determination of this protein at the patient side.[9]

Objective

TO PERFORM THE VALIDATION OF A NEW FELINE-SPECIFIC IMMUNOASSAY (Fuji Dri-Chem Immuno AU Cartridge vf-SAA) FOR IN-HOUSE SAA MEASUREMENT

Material & methods

An analytical validation including precision (Coefficients of Variations; CVs) and accuracy (linearity under dilution and recovery) was performed with the Fuji assay (Method A). These tests were also performed for comparative purposes with other two previously validated methods such as the SAA TIA, LZ-SAA[10] (Eiken Chemical Co., Ltd; analyser Olympus AU600; Method B) and other in-house methods, SAA VET test kit[11] (CUBE-VET analyser, Eurolyser Diagnostica GmbH; Method C) and LifeAssays® method (Method D). Correlations between the A, B and C methods were studied.

Twenty-five feline serum samples with different inflammatory diseases (mainly pancreatitis and panleukopenia) and 26 samples from non-inflammatory diseases were selected from our database for the clinical validation study.

Man-Whitney U test was used to investigate the significance between inflammatory and non-inflammatory disease cats' groups. Spearman correlation test was performed to assess correlation between data obtained by different methods.

Results & Discussion

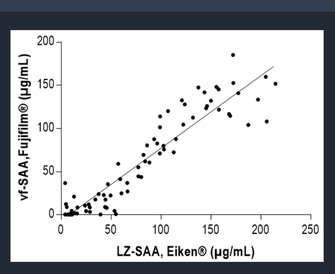
All methods showed high linearity after dilution and recovery rates between acceptable percentages (80-120%). Method A showed good precision in high, medium and low SAA concentrations (Cvs < 10% in all cases), comparable to Method B and better than the in-house methods C and D (Tables 1 and 2). (Further evaluations were not performed with the method D because it was not analytically robust).
The assay validated was highly correlated with the previously validated method (Figure 1) and was able to detect the different SAA concentrations between cats with and without inflammatory conditions in the overlap performance test (p< 0.001) (Figure 2).

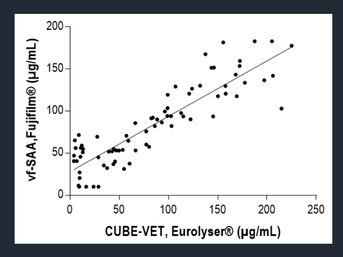
Table 1. Intra-assay repeatability of the assays for detection of a high (>100 µg/mL), medium (≈50 µg/mL) and low (≈25 µg/mL) concentrations of feline serum amyloid A protein.

Test	High (>100 µg/mL)	Medium (≈50 µg/mL)	Low (≈25 µg/mL)
Method A			
Mean ± SD	206.26 ± 12.5	64.21 ± 0.59	25.15 ± 0.53
CV (%)	6.07	0.93	2.12
Method B			
Mean ± SD	131.46 ± 1.39	64.44 ± 0.45	33.88 ± 0.43
CV (%)	1.06	0.71	1.28
Method C			
Mean ± SD	203.00 ± 12.55	60.40±2.00	27.68±15.86
CV (%)	6.19	3.33	12.23
Method D			
Mean ± SD	57.6 ± 6.50	20.60 ± 1.67	11.6 ± 0.89
CV (%)	11.29	8.12	7.71

Table 2. Inter-assay repeatability of the assays for detection of a high (>100 µg/mL), medium (≈50 µg/mL) and low (≈25 µg/mL) concentrations of feline serum amyloid A protein.

Test	High (>100 µg/mL)	Medium (≈50 µg/mL)	Low (≈25 µg/mL)
Method A			
Mean ± SD	210.95 ± 12.86	62.66 ± 2.30	26.83 ± 0.84
CV (%)	6.10	3.68	3.16
Method B			
Mean ± SD	128.25 ± 2.60	63.40 ± 2.79	35.07 ± 3.27
CV (%)	2.02	4.41	9.33
Method C			
Mean ± SD	204.57 ± 20.90	59.17 ± 13.10	41.75 ± 10.38
CV (%)	10.22	22.15	24.88
Method D			
Mean ± SD	48.5 ± 6.55	21.75 ± 2.87	14.00 ± 4.08
CV (%)	13.52	13.21	29.19





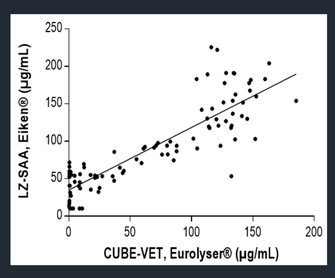


Figure 1. Representative graph of correlation test (n=79). Coefficient of correlation (r) between methods A and B (Fuji – Eiken) was 0.922 (equation, y=0.839x-6.623), between methods A and C (Fuji – EuroLyser) was 0.870 (equation, y=0.659x+27.9), and between methods B and C (Eiken – EuroLyser) was 0.889 (equation, y=0.8359x+34.77).

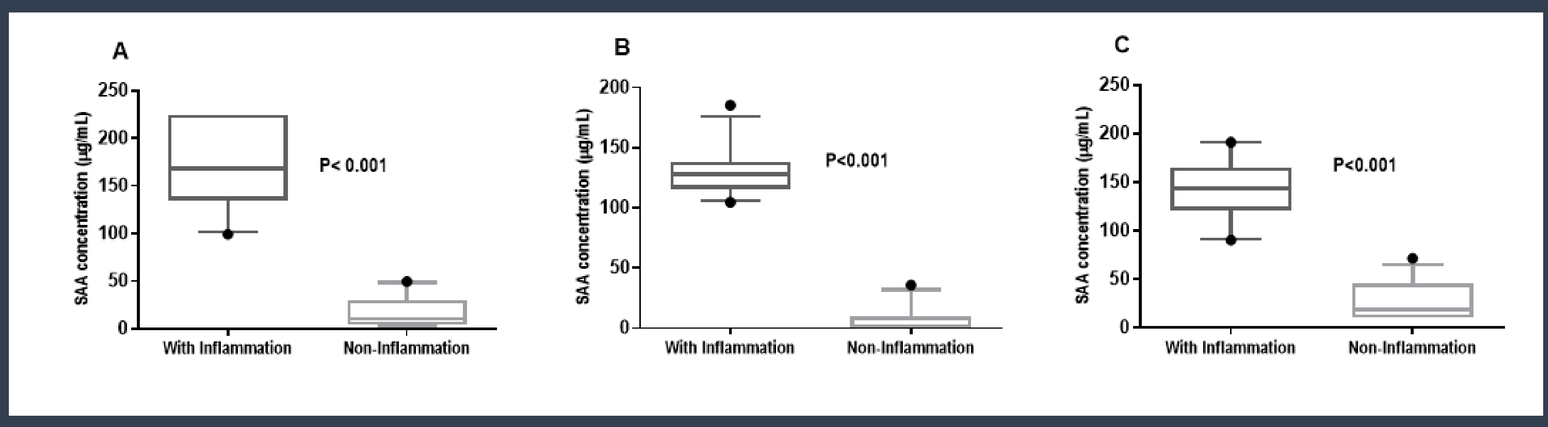


Figure 2. Serum amyloid A (SAA) in cats with (n=25) and without (n=26) inflammation as determined with FUJI DRI-CHEM IMMUNO AU CARTRIDGE vf-SAA, FUJIFILM (A), Eiken Chemical Co., Ltd; analyser Olympus AU600 (B) and CUBE-VET analyser, Eurolyser Diagnostica GmbH (C).

CONCLUSION

Based on the results the feline-specific immunoassay (Fuji Dri-chem Immuno AU Cartidge vf-SAA) is suitable for the measurement of SAA concentration in cats.

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