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New immunoassays for the detection of the most common feline serum amyloid A (SAA) variants

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Introduction

SAA is an acute phase protein and a sensitive marker of inflammation in cats. Under inflammatory conditions concentration of SAA in blood can increase more than 100-fold.

The concentration of SAA in blood is determined by immunoassays. It is important that antibodies used in immunoassays recognize all SAA forms present in blood. Several SAA variants were described in cats (Van Rossum et al, 2004) in which substitutions at positions 29, 45, 51, and 75 were the most common. **The goal of the study** was to develop prototype immunoassays that recognize SAA variants with substitutions at positions 29, 45, 51 and 75 as well as feline SAA with the reference sequence.

Methods

Feline SAA (GeneBank AF136718, used as a reference sequence) and four variants containing single amino acid substitutions I29/K29, Q45/R45, A51/V51 and N75/S75 were expressed in *E. coli* and purified by immunoaffinity chromatography.

Monoclonal antibodies FSA4050, FSA4071, FSA1173 and FSA1231 specific to feline SAA were from HyTest.

Sandwich immunoassays were performed in 96-well plates. Detection antibodies conjugated with europium chelate were was used as a label.

SAA concentrations in EDTA plasma samples from healthy cats (n=19) and cats with inflammation induced by surgery (n=21) were determined by developed immunoassays. Recombinant feline SAA with the reference sequence was used as a calibrator.

Results

Immunoassays for feline SAA

Two antibody pairs FSA4071 – FSA1173 (immunoassay 1) and FSA1231 – FSA4050 (immunoassay 2) were selected that recognized feline SAA with the reference sequence and four variants with about the same efficiency (Fig. 1).

The measurement ranges were 0.0002 – 0.1 μg/ml for both immunoassays (Fig. 2). Since SAA level in blood is above the measurement ranges, plasma samples should be diluted prior to concentration measurement.

Antibodies used in the immunoassay 1 poorly recognized SAA at room temperature. For this immunoassay incubation with calibrator and plasma samples should be performed at +37°C for 1 hour. For the immunoassay 2, incubation could be carried out either at room temperature or at +37°C. Immunoassay 2 demonstrated higher signals after long incubation time (1 hour), however incubation can be shortened to 15 minutes.

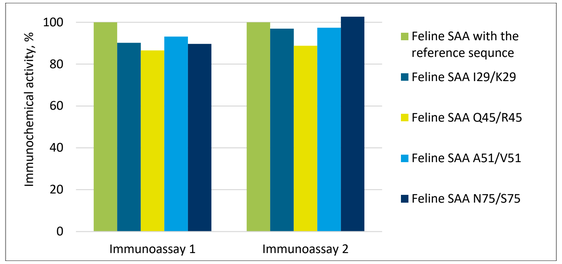

FIGURE 1. Cross-reactivity of immunoassays with feline SAA variants. Results are provided for SAA concentration 0.03 μg/ml. Signal obtained with feline SAA with the reference sequence was taken as 100%.

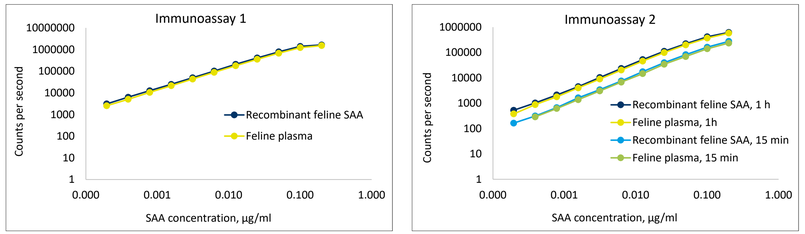

FIGURE 2. Dilution curves of recombinant feline SAA and pooled feline plasma. Plasma was initially diluted 400-fold. For immunoassay 1, incubation was performed at +37°C for 1 hour; for immunoassay 2, incubation was performed at room temperature for 15 minutes or 1 hour.

Dilution curves of feline plasma and recombinant feline SAA were parallel. This allows accurate measurement of SAA concentration in diluted plasma samples. What is important, recombinant feline SAA can be used as a calibrator for SAA quantification in plasma samples.

SAA concentration in feline plasma samples

Elevated SAA levels were observed in cats with inflammation compared to healthy cats. In healthy cats, median SAA concentration in plasma was 0.347 μg/ml (range 0.103-4.425 μg/ml) and 0.404 μg/ml (range 0.125-4.509 μg/ml) for immunoassays 1 and 2, respectively. In cats after surgery median SAA concentration was 189 μg/ml (range 23-330 μg/ml) and 191 μg/ml (range 22-355 μg/ml) for immunoassays 1 and 2, respectively (Fig. 3).

SAA concentrations determined by the two immunoassays in feline plasma samples demonstrated high correlation (r=0.99, p<0.05; Fig. 4).

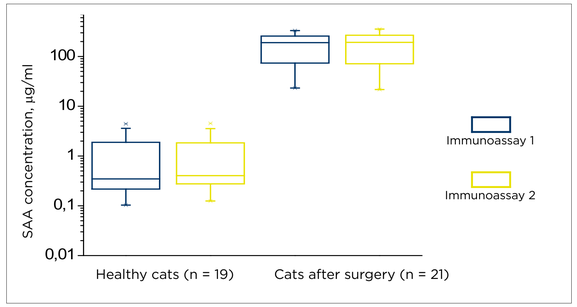

FIGURE 3. Ranges of plasma SAA concentrations in apparently healthy cats (n=19) and cats with inflammation induced by surgery (n=21). Results are displayed as a box-whisker plot. Horizontal lines indicate median values, boxes indicate values between the 25th and 75th percentiles, and whiskers indicate the minimum and maximum values.

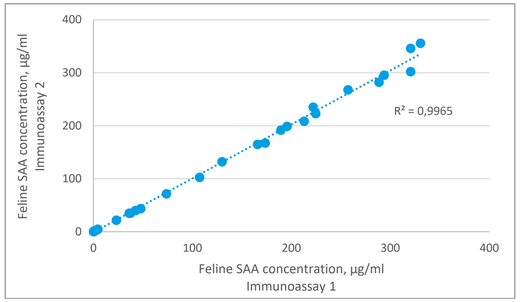

FIGURE 4. Correlation between SAA concentrations determined in 40 feline plasma samples by the developed immunoassays.

Conclusions

We developed two new prototype immunoassays for accurate measurement of the most common SAA variants in feline plasma samples. Immunoassay 2 would be suitable for development of a point-of-care assay as it can be carried out at room temperature and incubation time can be as short as 15 minutes. Recombinant feline SAA can be used as a calibrator in developed immunoassays.