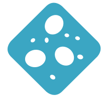
# TechNotes

HyTest

Clinical and Research Area | Veterinary | Inflammation 

# Feline, canine and equine serum amyloid A (SAA) is a marker of inflammation

Serum amyloid A (SAA) is a major acute phase protein in many species including humans beings, dogs, cats and horses. The level of SAA proteins in blood increases within just a few hours following the onset of various inflammatory stimuli. These include infection, trauma and surgery. As a result of its short half-life, the concentration of SAA decreases rapidly following the removal or elimination of the source of inflammation.

Whilst in circulation, SAA is associated with high-density lipoprotein (HDL). Several isoforms of SAA have been reported in each of the species that differ in several amino acid residues. In addition to acute phase isoforms, constitutive SAA isoform has been identified in both human beings and mice. Its expression is not elevated during acute phase response.

The SAAs are highly conserved across vertebrate species. Human SAA consists of 104 a.a.r. Meanwhile, canine (dog), feline (cat) and equine (horse) SAA contains an insertion of eight amino acids in the central part of the molecule as compared to human SAA. Canine and feline SAAs consist of 111 a.a.r. while equine SAA consists of 110 a.a.r. Further information regarding SAA can be found in comprehensive reviews (i.e. 1-3).

### SAA as a diagnostic marker

SAA is a sensitive marker of inflammation and tissue damage. In veterinary medicine, SAA measurements in blood might be used for the diagnosis of subclinical inflammation, the monitoring of treatment efficacy in animals with infections or inflammatory conditions, and the monitoring of patients who are undergoing surgery.

### Reagents for the development of reliable, species-specific SAA immunoassays

At HyTest, we provide several murine monoclonal antibodies that can be used for the development of immunoassays that enable the detection of feline, canine and equine SAA. We have tested these antibodies in sandwich immunoassays, direct ELISA and Western blotting, and they recognize SAA from animal serum samples. Furthermore, we also offer recombinant feline, canine and equine SAA proteins.

## Monoclonal antibodies specific to SAA

We provide several well-characterized anti-SAA MAbs for the detection of feline, canine and equine SAA. These MAbs were selected from a pool of over forty MAbs that were raised against human SAA, canine SAA, or synthetic peptides derived from canine SAA or human SAA. Specificities of the antibodies are shown in Table 1. Seven MAbs recognize feline SAA in direct ELISA and Western Blotting. Meanwhile, all MAbs recognize canine and equine SAA.

**Table 1. Specificities of anti-SAA MAbs.**

| Cat.# | MAb | Specificity | | |
|---|---|---|---|---|
| | | Canine SAA | Feline SAA | Equine SAA |
| 4VS4 | SAA11 | + | + | + |
| | SAA19 | + | + | + |
| | SAA21 | + | + | + |
| | VSA2 | + | - | + |
| | VSA31 | + | + | + |
| | VSA34 | + | + | + |
| | VSA38 | + | + | + |
| | VSA43 | + | +/- | + |
| 4SA11 | VSA6 | + | - | + |
| | VSA25 | + | + | + |


1. **Jacobsen, S. and Andersen, P.H.** The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. Equine Vet Educ, 2007, 19: 38–46.

2. **Ceron J.J. et al.** Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet Clin Pathol, 2005, 34:85-99.

3. **Yamada T.** Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin Chem Lab Med, 1999, 37:381-388.

### Development of sandwich immunoassays for feline, canine and equine SAA

For the development of a sandwich immunoassay for the measurement of SAA in cat serum samples we recommend three MAb combinations (see Table 2). A dilution curve of recombinant feline SAA for the MAb combination SAA19–VSA34 is provided in Figure 1A.

A high level of sequence homology between dog and horse SAA proteins allows the use of the same antibody combinations for the detection of SAA in both species. All of our antibodies detect both dog and horse SAA. Based on our tests, five antibody combinations (see Table 2) show high sensitivity and specificity in sandwich immunoassays. Dilution curves of recombinant canine (B) and equine (C) SAA for the MAb combination VSA2–VSA38 are provided in Figure 1.

| Capture | Detection |
|---|---|
| Assay for cats, dogs and horses | |
| SAA19 | VSA34 |
| SAA21 | VSA34 |
| VSA25 | VSA31 |
| Assay for dogs and horses | |
| VSA2 | VSA38 |
| VSA2 | VSA31 |
| VSA6 | VSA38 |
| VSA25 | VSA31 |
| VSA38 | VSA43 |

**Table 2. The most sensitive capture-detection pairs.** Data is based on the results obtained using our in-house time-resolved fluorescence immunoassay.

Figure 2 illustrates the detection of SAA in serum samples that were obtained from cats, dogs and horses. SAA immunoreactivity in serum samples obtained from animals with inflammatory diseases was considerably higher as compared to SAA immunoreactivity in normal serum samples.

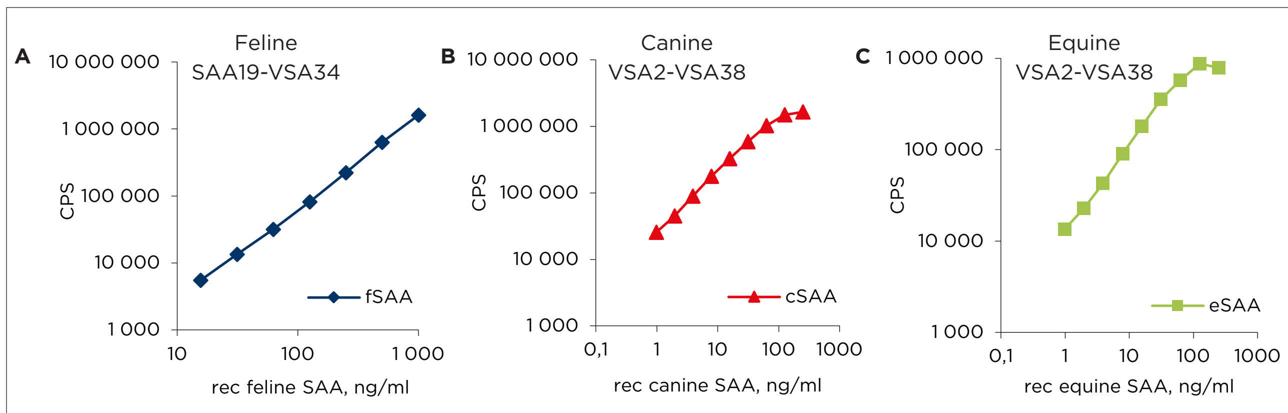



**Figure 1. Dilution curves of recombinant feline (A), canine (B) and (C) equine SAA.** Antibody combinations are indicated in the figure. (A): Plate wells coated with SAA19 were blocked with 2.5% sodium caseinate at 37°C for one hour. Recombinant feline SAA (Cat #8FS5) was serially diluted in Tris-buffer that contained 0.01% CHAPS and 1:1000 normal serum and was incubated in plate wells at room temperature for one hour. VSA34 ($Eu^{3+}$ labeled), was diluted in Tris-buffer that contained 0.01% CHAPS. The same buffer was used for plate washing. (B-C): Plate wells coated with VSA2 were blocked with a buffer containing 1% casein and 0.05% Tween 20 at 37°C for one hour. The recombinant canine SAA (Cat.# 8CS4) and equine SAA (Cat.# 8ES6) were serially diluted in the same buffer and incubated in the coated plate wells at 37°C for one hour. MAb VSA38 ($Eu^{3+}$ labeled) was used as a detection antibody.

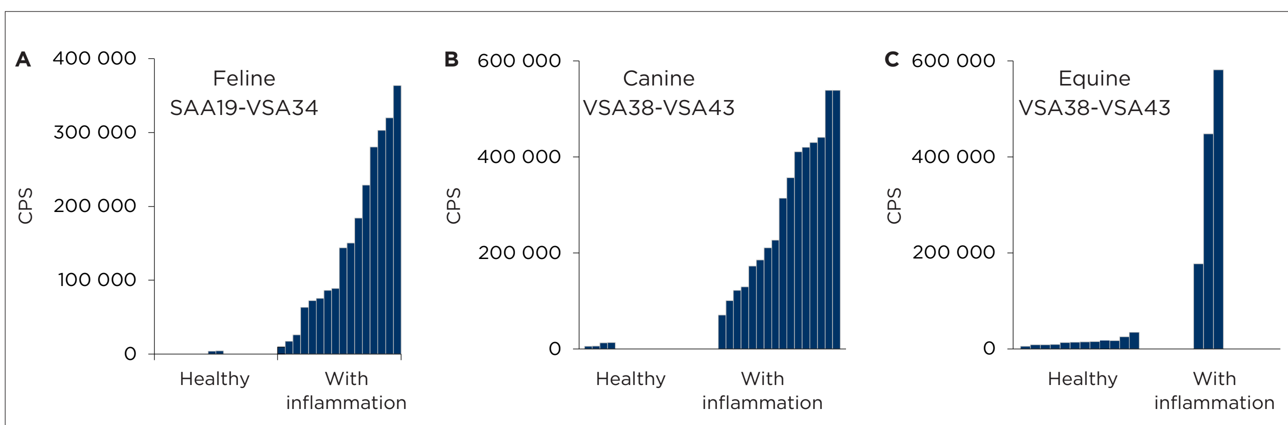



**Figure 2. Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased cats (A), dogs (B) and horses (C).** Antibody combinations are indicated in the figure. Protocols were as described in the caption of Figure 1. In A, the serum samples were diluted 800-fold in Tris-buffer containing 0.01% CHAPS. In B-C, the serum samples from healthy animals were diluted 50-fold with a blocking buffer that contained 1% casein and 0.05% Tween 20. Samples from diseased animals were either diluted 2000-fold (dog serum) or 1000-fold (horse serum).

### Development of a single immunoassay for feline, canine and equine SAA

The assays suitable for the detection of feline SAA are also suitable for the detection of canine and equine SAA (see Table 2). Figure 4 illustrates the detection of SAA in serum samples that were obtained from dogs (A) and horses (B) using the MAb combination SAA19–VSA34. SAA immunoreactivity in serum samples obtained from diseased animals was considerably higher as compared to SAA immunoreactivity in normal serum samples. Results of feline serum samples testing using the same MAb combination are provided in Figure 2.

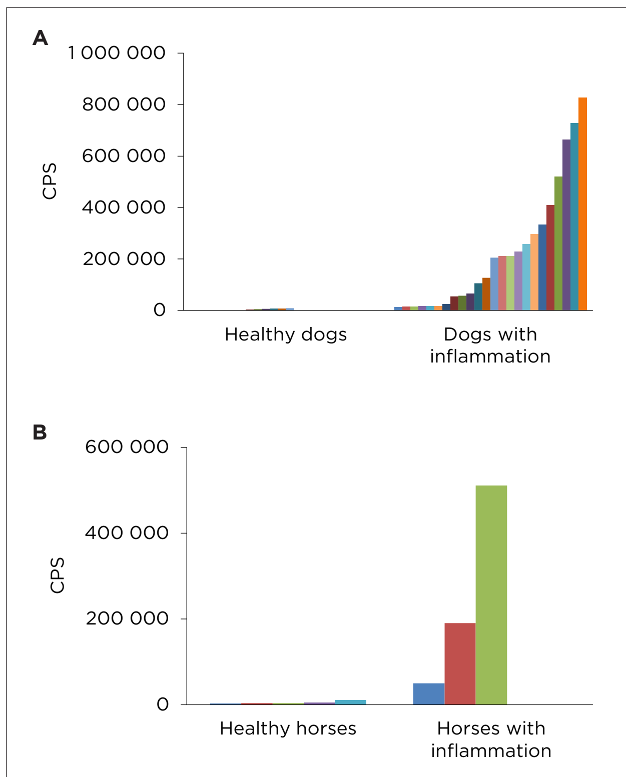



**Figure 4. Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased dogs (A) and horses (B) detected by using the MAb combination SAA19–VSA34.** Protocol was as described in the caption of Figure 1. Dog and horse serum samples were diluted 500-fold and 800-fold respectively in Tris-buffer containing 0.01% CHAPS.

### Important notes for assay design

It is known from earlier publications that human SAA adsorbs non-specifically onto polystyrene surfaces of microtiter plates (4, 5). We observed that SAA from other species (dogs, cats and horses) behaves similarly to human SAA and preferentially adsorbs from serum onto the plate's surface. Therefore, when developing a SAA immunoassay in microtiter plates, it is important to prevent non-specific binding of SAA to the wells of a plate. A plate blocking procedure and antigen dilution buffer should be optimized to ensure that SAA non-specific binding to the plate wells is suppressed.

According to our data, casein appears to be an effective blocking agent for SAA immunoassays. For the MAb combinations SAA19–VSA34 and SAA21–VSA34, either the Tris buffer containing 1% casein or 2.5% sodium caseinate was used for microliter plate blocking.

For the MAb combinations VSA2–VSA38, VSA2–VSA31, VSA6–VSA38, VSA25–VSA31 and VSA38–VSA43, Tris buffer containing 1% casein and 0.05-0.1% Tween 20 was the most efficient solution for microtiter plate blocking.

Also, we observed that the non-ionic surfactant Tween 20, which is a regular component of antigen dilution and washing buffers, suppressed the signal in MAb combinations SAA19–VSA34 and SAA21–VSA34. Therefore, for these two combinations, we substituted Tween 20 with the zwitterionic surfactant CHAPS (0.01%) in antigen dilution buffer and washing buffer.

Please note that the suggested solid phase blocking procedure and assay buffer composition were optimized for HyTest's in-house immunoassays. Therefore, other conditions could demonstrate an improved performance in the immunoassays of our customers than those described here.

**4. Marhaug, G.** Three assays for the characterization and quantitation of human serum amyloid A. Scand J Immunol, 1983, 18:329–338.

**5. Časl, MT and Grubb, A.** A rapid enzyme-linked immunosorbent assay for serum amyloid A using sequence-specific antibodies. Ann Clin Biochem, 1993, 30: 278-286.

### Direct ELISA

All MAbs recognize canine, feline (with the exception of MAbs VSA2, VSA6 and VSA43) and equine SAA proteins coated onto the wells of polystyrene 96-well plates. The titration curves of four MAbs obtained with the recombinant canine SAA are provided in Figure 5.

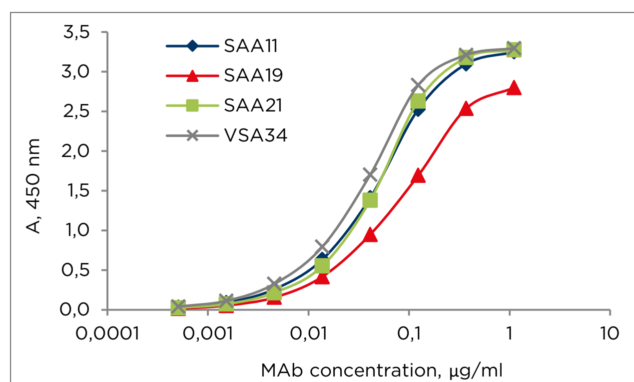



**Figure 5. Titration curves of the MAbs obtained with recombinant canine SAA.** A microtiter plate was coated with 0.05 µg of recombinant canine SAA in PBS. MAbs were serially diluted in PBS, pH 7.4, containing 0.1% Tween 20. HRP-conjugated anti-mouse IgG (Fc specific) was used as a secondary antibody. o-Phenylenediamine was used as a substrate.

## Recombinant canine, feline and equine SAA

HyTest provides recombinant canine (121 a.a.r.), feline (121 a.a.r.) and equine (120 a.a.r.) SAA proteins expressed in *E. coli*. Recombinant SAA proteins contain an additional affinity tag that consists of 10 a.a.r. on the N-terminus of the SAA molecule. Amino acid sequences of recombinant SAA proteins (without tag) are provided in Figure 6. Note, recombinant feline SAA is also available a a non-tagged version

Recombinant SAAs are purified to homogeneity using several chromatographic steps. The purity of recombinant SAAs is assessed by densitometry following SDS-electrophoresis in reducing conditions and exceeds 95%. Figure 7 shows the SDS-PAGE of purified canine, feline and equine SAA.

Canine SAA — kDa: 130, 70, 55, 25, 15, 10
Feline SAA — kDa: 130, 70, 55, 25, 15, 10
Equine SAA — kDa: 130, 70, 55, 25, 15, 10

**Figure 7. Tricine-SDS-PAGE of recombinant canine, feline and equine SAA in reducing conditions.** 10 µg of protein was loaded per lane. Gels were stained using Coomassie Brilliant Blue R-250.

| | 1 | | | | | | | | | 10 | | | | | | | | | | 20 | | | | | | | | | | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| canine SAA | Q | W | Y | S | F | V | S | E | A | A | Q | G | A | W | D | M | W | R | A | Y | S | D | M | R | E | A | N | Y | K | N |
| feline SAA | E | W | Y | S | F | L | G | E | A | A | Q | G | A | W | D | M | W | R | A | Y | S | D | M | R | E | A | N | Y | I | G |
| equine SAA | | L | L | S | F | L | G | E | A | A | R | G | T | W | D | M | I | R | A | Y | N | D | M | R | E | A | N | Y | I | G |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 31 | | | | | | | | | 40 | | | | | | | | | | 50 | | | | | | | | | | 60 |
| canine SAA | S | D | K | Y | F | H | A | R | G | N | Y | D | A | A | Q | R | G | P | G | G | A | W | A | A | K | V | I | S | D | A |
| feline SAA | A | D | K | Y | F | H | A | R | G | N | Y | D | A | A | Q | R | G | P | G | G | A | W | A | A | K | V | I | S | D | A |
| equine SAA | A | D | K | Y | F | H | A | R | G | N | Y | D | A | A | K | R | G | P | G | G | A | W | A | A | K | V | I | S | D | A |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 61 | | | | | | | | | 70 | | | | | | | | | | 80 | | | | | | | | | | 90 |
| canine SAA | R | E | N | S | Q | R | I | T | D | L | L | R | F | G | D | S | G | H | G | A | E | D | S | K | A | D | Q | A | A | N |
| feline SAA | R | E | N | S | Q | R | V | T | D | F | F | R | H | G | N | S | G | H | G | A | E | D | S | K | A | D | Q | E | A | N |
| equine SAA | R | E | N | F | Q | R | F | T | D | R | F | S | F | G | G | S | G | R | G | A | E | D | S | R | A | D | Q | A | A | N |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 91 | | | | | | | | | 100 | | | | | | | | | | 110 | | | | | | | | | | |
| canine SAA | E | W | G | R | S | G | K | D | P | N | H | F | R | P | A | G | L | P | D | K | Y | | | | | | | | | |
| feline SAA | E | W | G | R | S | G | K | D | P | N | H | Y | R | P | E | G | L | P | D | K | Y | | | | | | | | | |
| equine SAA | E | W | G | R | S | G | K | D | P | N | H | F | R | P | H | G | L | P | D | K | Y | | | | | | | | | |

**Figure 6. Sequence alignment of HyTest's recombinant canine, feline and equine SAA.** Identical a.a.r. are highlighted in blue. Recombinant proteins contain an additional 10 a.a.r. on the N-terminus.

## Ordering information

### MONOCLONAL ANTIBODIES

| Product name | Cat. # | MAb | Subclass | Remarks | |
|---|---|---|---|---|---|
| Serum amyloid A (SAA), animal | 4VS4 | VSA2 | IgG1 | EIA, WB. Specific to canine and equine SAA. | |
| | | SAA11 | IgG2b | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | SAA19 | IgG2a | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | SAA19cc | IgG2a | *In vitro*, EIA, WB. Specific to canine, feline and equine SAA. | New! |
| | | SAA21 | IgG2b | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | SAA21cc | IgG2b | *In vitro*, EIA, WB. Specific to canine, feline and equine SAA. | New! |
| | | VSA31 | IgG2a | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | VSA31cc | IgG2a | *In vitro*, EIA, WB. Specific to canine, feline and equine SAA. | New! |
| | | VSA34 | IgG2b | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | VSA34cc | IgG2b | *In vitro*, EIA, WB. Specific to canine, feline and equine SAA. | New! |
| | | VSA38 | IgG2a | EIA, WB. Specific to canine, feline and equine SAA. | |
| | | VSA38cc | IgG2a | *In vitro*, EIA, WB. Specific to canine, feline and equine SAA. | New! |
| | | VSA43 | IgG2b | EIA, WB. Specific to canine and equine SAA. | |
| Serum amyloid A (SAA), human | 4SAA11 | SAA11 | IgG1 | EIA, WB, Specific to canine canine. | |
| | | SAA6 | IgG1 | EIA, WB, Specific to canine canine. | |
| | | SAA15 | IgG1 | EIA, WB, Specific to canine canine. | |
| | | VSA6 | IgG1 | EIA, WB. Specific to canine and equine SAA. | |
| | | VSA25 | IgG1 | EIA, WB. Specific to canine, feline and equine SAA. | |

### ANTIGENS

| Product name | Cat. # | Purity | Source | |
|---|---|---|---|---|
| Serum amyloid A (SAA), canine, recombinant | 8CS4 | >95% | Recombinant | |
| Serum amyloid A (SAA), equine, recombinant | 8ES6 | >95% | Recombinant | |
| Serum amyloid A (SAA), feline, recombinant | 8FS5 | >95% | Recombinant | |
| Serum amyloid A (SAA), feline, recombinant, non-tagged | 8FT7 | >95% | Recombinant | New! |

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