Monoclonal Serum Amyloid A Antibodies Applicable to Human and Veterinary Diagnostic Immunoassays with Wide Detection Ranges

S. Eklin[1], T. Hämäläinen[1], M. Mattila[1], W. Sun[2], S. Tiitinen[1]

[1] Medix Biochemica, Espoo, Finland [2] Medix Biochemica, Shanghai, China

Medix Biochemica

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SAA as a diagnostic marker

Serum amyloid A (SAA) is a family of proteins synthesized in response to inflammatory signals. Humans have four SAA isoforms, of which SAA1, and possibly SAA2, are considered to be clinically relevant. SAA concentrations in blood increase within a few hours after the onset of inflammation as part of the acute-phase immune response. Normal levels vary from <2–8 mg/L and increase 1000-fold during acute inflammation. Like CRP, SAA can therefore be used as an indicator of microbial infection and various inflammatory conditions. As SAA proteins are highly conserved in vertebrates, SAA measurements can also be used in veterinary diagnostics, where SAA has been shown to be superior to CRP as a diagnostic marker.[1]

SAA has been shown to have better sensitivity and diagnostic efficiency than CRP in various human inflammatory conditions.[2–5] Combined use of SAA and CRP can improve diagnosis and monitoring of inflammatory disease and enable differentiation between bacterial and viral infections,[3,5–8] specifically because serum SAA concentrations increase substantially more than CRP during viral infections.[6,7] Recently, SAA has also been shown to have prognostic value for patients with COVID-19.[9–13]

We have developed eight mouse monoclonal antibodies against human SAA. In addition to human diagnostics, these antibodies can also be applied in veterinary diagnostics.

Materials & Methods

Eight mouse monoclonal antibodies (mAbs) against human SAA1 were developed, designated as: Anti-h SAA 2201 (#100279), 2203 (#100289), 2205 (#100802), 2208 (#100805), 2209 (#100806), 2211 (#100808), 2212 (#100809), and 2213 (#100810).

Binding specificities of these antibodies were studied in fluorescence-based immunoassays (FIA).

Selected antibody pairs were tested in in vitro diagnostic (IVD) applications for differences in linear measuring range and ability to measure SAA in clinical samples: immunoturbidimetric assay (IT), lateral flow assay (LF), and FIA. Sensitivity for veterinary antigens was measured with FIA. Latex-enhanced immunonephelometry assay (Siemens N latex SAA Assay) and BioSite ELISA cat KSP-225 were used as reference methods.

Overlapping peptide scan was applied to identify continuous epitopes for the antibodies.

Binding specificities of SAA antibodies

Binding specificities of the eight antibodies were studied in FIA with native human SAA, and recombinant SAA proteins: human SAA1, SAA2, SAA4; and equine, feline and canine SAA1 (Figure 1).

All antibodies recognized native human SAA and recombinant human SAA1, and antibodies 2205 and 2208 were specific to these SAA forms. Antibodies with more diverse recognition profiles recognized feline (2201, 2209, 2212, 2213), canine (2201, 2209, 2211, 2213) and equine (2201, 2203, 2209, 2211, 2212, 2213) SAA in addition to the human SAA forms (Figure 1). None of the antibodies recognized bovine SAA.

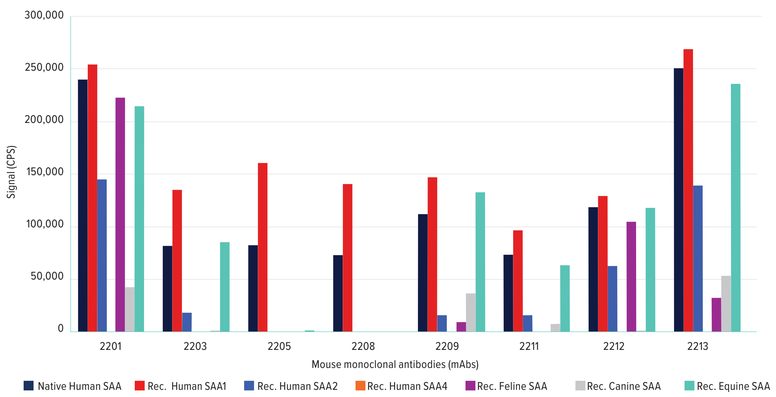

Figure 1. *SAA antigen recognition profile of the eight antibodies in direct coating FIA.*

Measuring range of SAA in clinical samples

In IT, the antibody pair 2201 + 2211 showed the widest linear measuring range. Forty-three clinical samples in the range of 4 to 307 mg/L were measured with the selected pair, and the results showed strong correlation (0.947) with the reference (Figure 2).

In LF, the widest linear measuring range was achieved with the antibody pair 2201 + 2203. This pair showed excellent performance in the 42 clinical samples tested, with a correlation coefficient of 0.976 (Figure 3).

In sandwich FIA, the widest linear measuring range was achieved with the human SAA1 specific antibody 2205 (capture) paired with 2212 (label). The clinical samples were diluted 1:10 for FIA measurements. The SAA measurements on the 2205 + 2212 pair also correlated strongly with the reference method (R2=0.9787) (Figure 4).

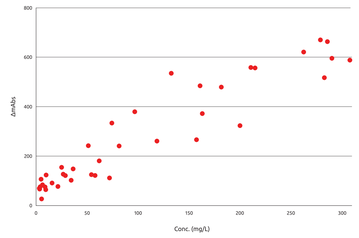

Figure 2. *IT assay results for the antibody pair with the widest measuring range (2201 + 2211).*

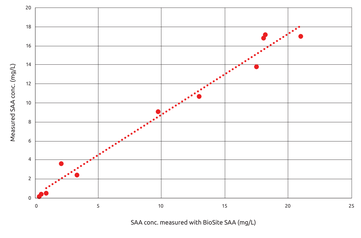

Figure 4. *Sandwich FIA results for the antibody pair 2205 (capture) + 2212 (label).*

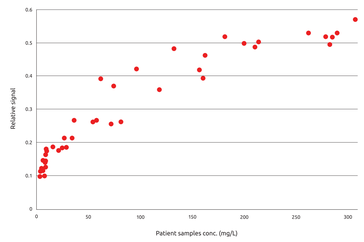

Figure 3. *LF results for the antibody pair with the widest measuring range (2201 + 2203).*

Veterinary diagnostics

Many of these antibodies also recognized feline, canine, or equine SAA (Figure 1), and therefore they can be used in veterinary diagnostics. The pair 2211 (capture) + 2209 (detection) showed the best sensitivity for both canine and equine recombinant antigens (Figure 5).

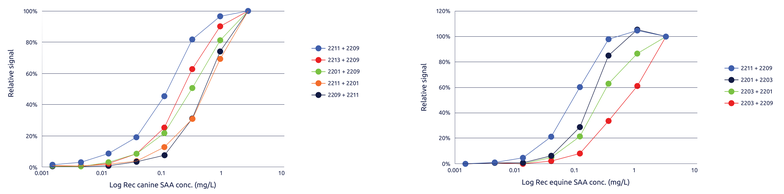

Figure 5. *Sensitivity for veterinary antigens (canine and equine) in sandwich FIA.*

Epitope identification

Linear epitopes were identified for three antibodies: 2205, 2208 and 2212. The epitopes were located in turn structures of the SAA1 protein: 2212 in an N-terminal turn, and both 2205 and 2208 in a C-terminal turn with overlapping epitopes in a region unique to human SAA1. Antibodies 2201, 2203, 2209, 2211, and 2213 did not bind to linear peptides, indicating binding to conformational epitopes.

Conclusions

These results demonstrate that our monoclonal SAA antibodies can be used to develop diagnostic assays with wide detection ranges for measuring SAA levels in human clinical samples. The results show high specificity and strong correlation with the reference methods.

The results also indicate that the best combination of antibodies in an IVD assay is dependent on the type of application and, consequently, the selection of antibodies should be based on the methods used. In addition to human diagnostics, the developed SAA antibodies can also be applied in veterinary diagnostics for highly sensitive measurement of equine, feline, and canine SAA.

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Medix Biochemica Group
Klovinpellontie 3, FI-02180 Espoo, Finland
medix@medixbiochemica.com • www.medixbiochemica.com