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Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations ☆

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Abstract

The aim of the present study was to evaluate whether equine serum amyloid A (SAA) concentrations could be measured reliably with a turbidometric immunoassay (TIA) developed for use with human serum. Intra- and inter-assay imprecision were evaluated by multiple measurements on equine serum pools. Assay inaccuracy was determined by linearity under dilution. The assay was subsequently used for measuring SAA concentrations in clinically healthy horses, horses with inflammatory diseases, horses with non-inflammatory diseases, and in horses before and after castration. In pools with low, intermediate and high SAA concentrations, the intra-assay imprecisions were 24.4%, 1.6% and 2.1%, and the inter-assay imprecisions were 33.2%, 4.6% and 6.5%. Slight signs of inaccuracy were observed, but these inaccuracies were negligible when considering the large dynamic range of the SAA response. The assay was able to detect the expected difference in SAA levels in different groups of horses. It was also able to demonstrate the expected dynamic changes in SAA after castration. In conclusion, equine SAA concentrations can be measured reliably using the TIA designed for human SAA.

Keywords: Acute phase protein; Horse; Immunoassay; Serum amyloid A; Test validation

1. Introduction

The protein serum amyloid A (SAA) has been identified as a major acute phase protein in humans and several veterinary species including the horse (Malle and de Beer, 1996; Kajikawa et al., 1999; Hultén and Demmers, 2002; Jacobsen et al., 2004). Serum concentrations of SAA are low in healthy horses but increase rapidly to very high levels in response to inflammation and tissue damage (Pepys et al., 1989; Nunokawa et al., 1993; Hultén et al., 2002). It has been suggested that concentrations of SAA reflect underlying disease activity and may thus be used, for example, to monitor changes in disease states and responses to therapy (Mozes et al., 1989; Hultén and Demmers, 2002; Jacobsen et al., 2004).

Several methods for measuring equine SAA have been developed, including an ELISA (Hultén et al., 1999b), slide reversed passive latex agglutination test (Wakimoto, 1996), single radial immunodiffusion (Nunokawa et al., 1993), latex agglutination immunoturbidometric assay (Stoneham et al., 2001) and electroimmunoassay (Pepys et al., 1989; Chavatte et al., 1992). Moreover, an ELISA developed for use in multiple species including the horse is commercially available (Tridelta Development Ltd.).

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To facilitate wide-spread use of SAA measurements in routine diagnosis, commercially available, rapid and preferably automated assays are likely to be helpful. Quantitative immunoaggregation assay are widely used in this respect due to their inherent simplicity, fast reaction times, often excellent precision, and ease of automation. A turbidometric assay (TIA) using the latex agglutination principle for measuring human SAA has been developed and is commercially available (LZ test SAA, Eiken Chemical Co. [Eiken SAA TIA]). The principle of the analysis is binding of SAA to a latex conjugated mixture of rabbit polyclonal and mouse monoclonal anti-human SAA antibodies to form a precipitate that is measured turbidometrically. To the authors' knowledge, a commercially available automated TIA for the determination of equine SAA has never previously been evaluated.

The aim of the present study was to evaluate the reliability of the Eiken SAA TIA for measuring equine serum SAA concentrations by investigating (1) assay characteristics (imprecision, inaccuracy, and detection limit [DL]), and (2) the ability of the assay to detect the expected difference in SAA levels between clinically healthy horses and horses suffering from inflammatory and non-inflammatory diseases, as well as the ability to monitor changes in SAA levels over time.

2. Materials and methods

2.1. SAA analysis

The Eiken SAA TIA (lot 470077) developed for measuring human serum SAA concentrations was used for heterologous determination of SAA in equine serum samples. The analyses were performed using an automated analyser (ADVIA 1650 Chemistry System, Bayer) according to the manufacturer's instructions with one exception: samples initially determined to contain more than 250 mg/L were re-run with a dilution of 1:3, as the assay had shown optimal performance below 250 mg/L in a preliminary study (data not shown). The re-run procedure was performed as an integral part of the assay conditions on the automated analyser. The calibration curve was made using a human calibrator supplied with the kit (lot 45002).

2.2. Assay characteristics of the TIA

Intra- and inter-assay variation was determined as the coefficient of variation (CV) from the mean and SD of 7–10 and 6–8 replicate determinations, respectively (Table 1), of three serum pools containing low, intermediate or high SAA concentrations (approximately 0.6, 57 and 772 mg/L, respectively, as determined by the Eiken SAA TIA). For the determination of inter-assay variation the pools were stored at −80 °C in aliquots, and for each analytical run only the aliquots needed were thawed in order to prevent potential variation due to repeated freeze-thaw episodes.

Table 1
Intra- and inter-assay variation in determination of serum amyloid A (SAA) concentrations in equine serum samples

Comparison	No. of samples	SAA concentration (mg/L)		Coefficient of variation (%)
		Mean	SD	
Intra-assay[a]	10	0.67	0.16	24.4
	10	55.48	0.89	1.6
	7	771.23	16.13	2.1
Inter-assay[a]	6	0.48	0.16	33.2
	6	60.85	2.78	4.6
	8	773.80	50.48	6.5

[a] Based on replicate determinations of the same serum pool.

Inaccuracy was investigated by evaluating linearity under dilution. Duplicate determinations of SAA concentrations were made from a serum pool with high concentrations of SAA diluted 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% using isotonic NaCl.

The DL was determined from 20 replicate determinations of isotonic NaCl.

2.3. Animals and samples

A total of 47 samples was analysed. The control samples originated from 18 clinically healthy horses and were obtained during routine health inspection at a riding school. The patient samples originated from 21 equine patients presented at the Large Animal Teaching Hospital at the Royal Veterinary and Agricultural University, Copenhagen, Denmark. All patients underwent clinical examination and standard blood biochemical and haematological testing. Based on the final diagnosis the patients were assigned to three groups: horses suffering from diseases characterised by acute or subacute inflammation ($n = 12$), horses suffering from non-inflammatory disease ($n = 6$), and healthy horses undergoing elective surgery (castration, $n = 3$).

Horses in the inflammation group were diagnosed with peritonitis, infectious arthritis/tenovaginitis, pleuritis, acute airway infection, botryomycosis, and abscessation. Two of the horses in this group had blood sampled twice: in one horse blood samples were obtained on days 1 and 3 after surgical resection of a large botryomycotic process on the chest, and in one horse blood samples were obtained before and one day after drainage of an abscess. Horses in the non-inflammatory group suffered from hyperlipaemia, non-inflammatory preputial oedema, acute liver failure, bilateral retinal detachment,