The Calibration of VMRD Serum Amyloid A (SAA)

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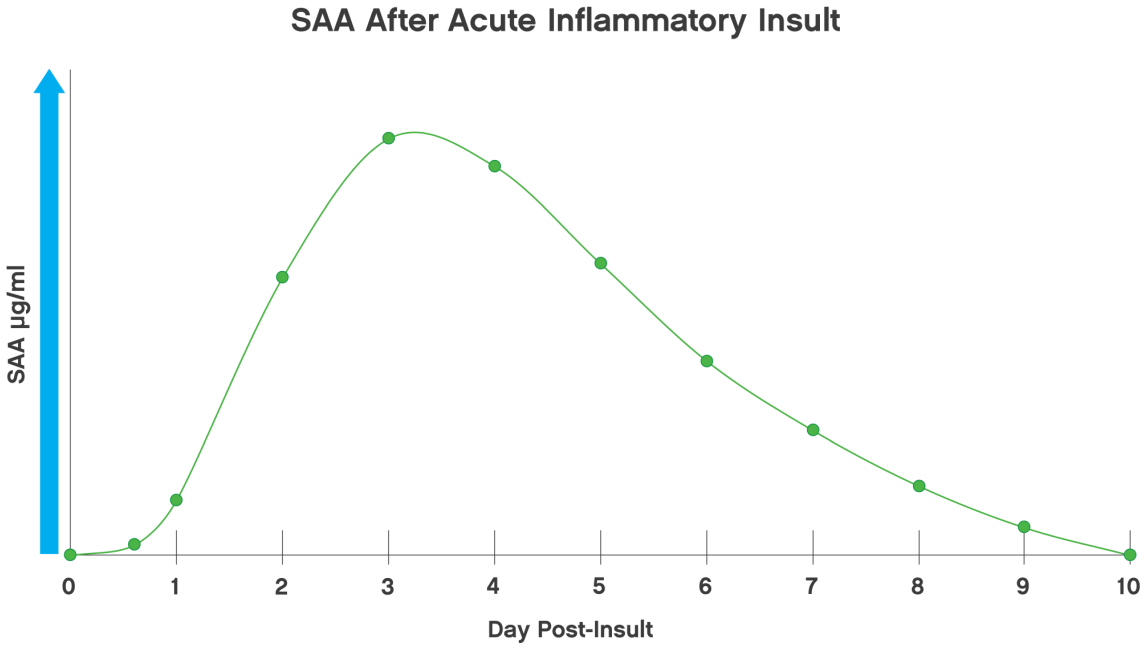
One of the key advantages for using VMRD's SAA test for equine and feline is the accuracy and consistency provided by the calibration system.

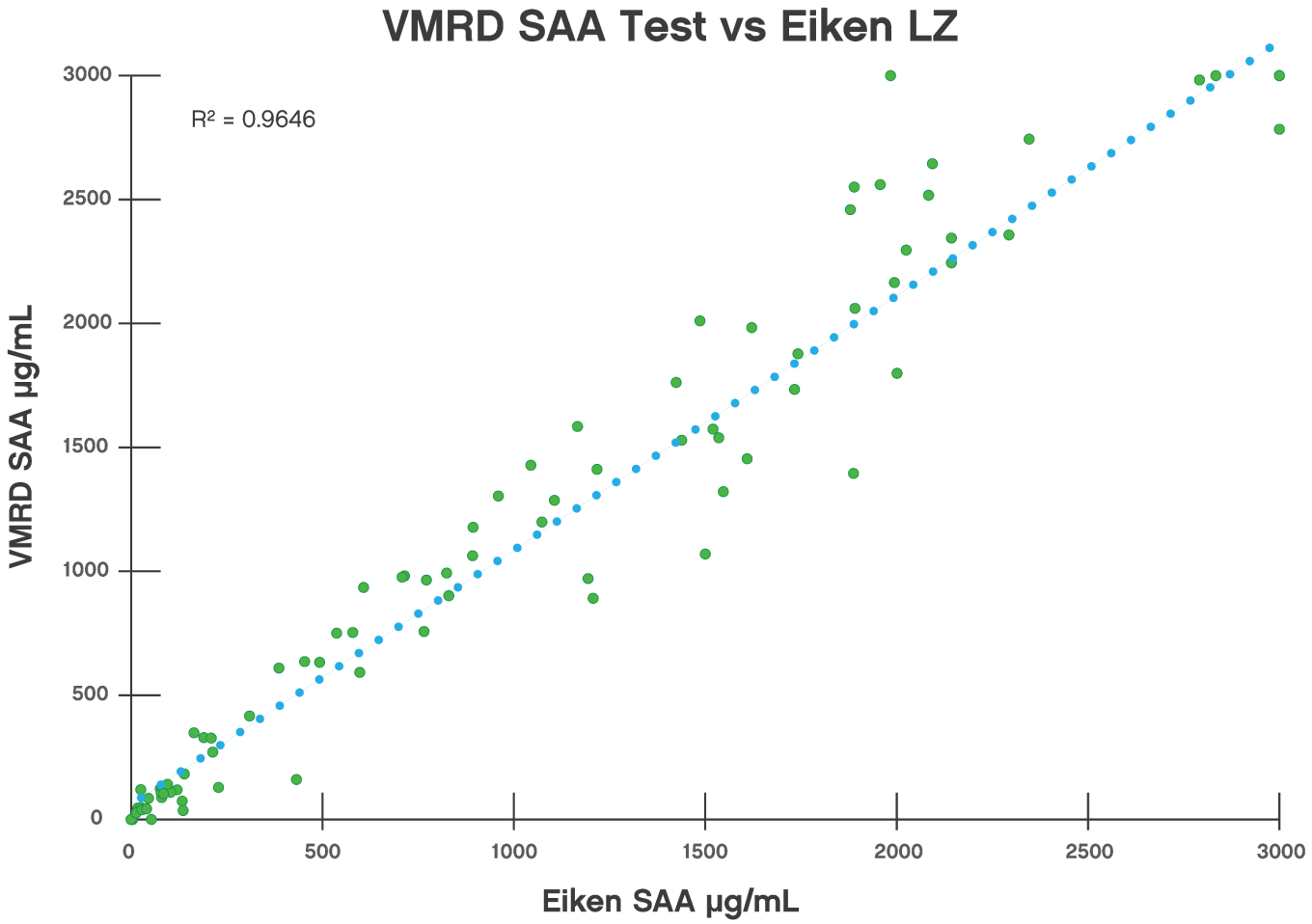
SAA is an "intrinsically disordered" protein.

SAA exists in varying configurations and changes structure frequently, including binding with cholesterol in the bloodstream, making quantification a challenge. Different assays have different components that affect recognition of SAA, therefore it is not unexpected that absolute numbers may vary between assays.[1] This is very similar to how reference ranges for biochemistry values often vary between labs and different machines when running standard bloodwork. Even if specific values may vary, the overall trend observed should be the same with SAA increasing after an acute systemic inflammatory insult (such as infection) and decreasing as the issue resolves.



The reference standard Eiken LZ-SAA is used for calibration of VMRD SAA.

There is no existing test that can provide exact quantitation of SAA, therefore it is necessary to understand the reference standard and its importance. During development of VMRD SAA, we chose to calibrate against the Eiken LZ-SAA assay as it has historically been considered the "gold standard" [2] used in most veterinary publications[3-31], and specifically validated for equine[32] and feline[33] samples. As intended, the equine VMRD SAA assay correlates very well with Eiken LZ-SAA, demonstrated in the independent validation study performed by Dr. Nicola Pusterla at UC Davis. [34]



(Data for graph adapted from Karam *et al.*[34])

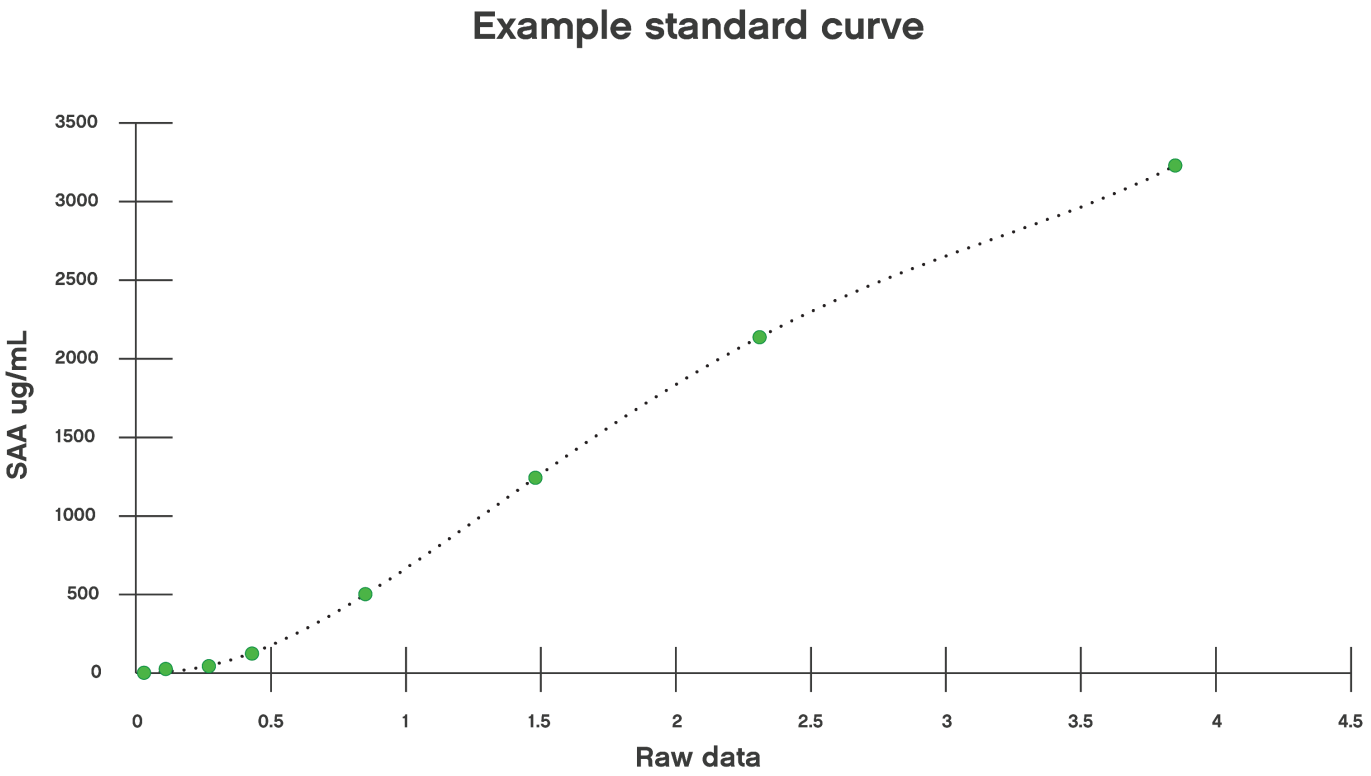
At the time of this article, Eiken stopped producing LZ-SAA and switched to a different assay for the veterinary market (VET-SAA) that could also be used for canine samples. This naturally led to a change in the assay used by university labs such as Cornell and the University of Miami to Eiken VET-SAA. In our experience, and that of others, VET-SAA results are often half the values seen with LZ-SAA. This may be related to the fact that at least 3 isoforms of SAA exist in horses[3,] and our understanding is that VET-SAA only recognizes 1 of these (SAA-1). Since use of the LZ-SAA assay has been much more extensively documented than VET-SAA, and the observed results between the two have been so different, VMRD elected to continue calibrating to LZ-SAA so that we could maintain the wide range of results historically found in horses. This enables more effective monitoring of trends as a broader range allows a greater change in SAA value associated with changes in clinical condition.

Every batch of VMRD SAA is calibrated for greatest consistency

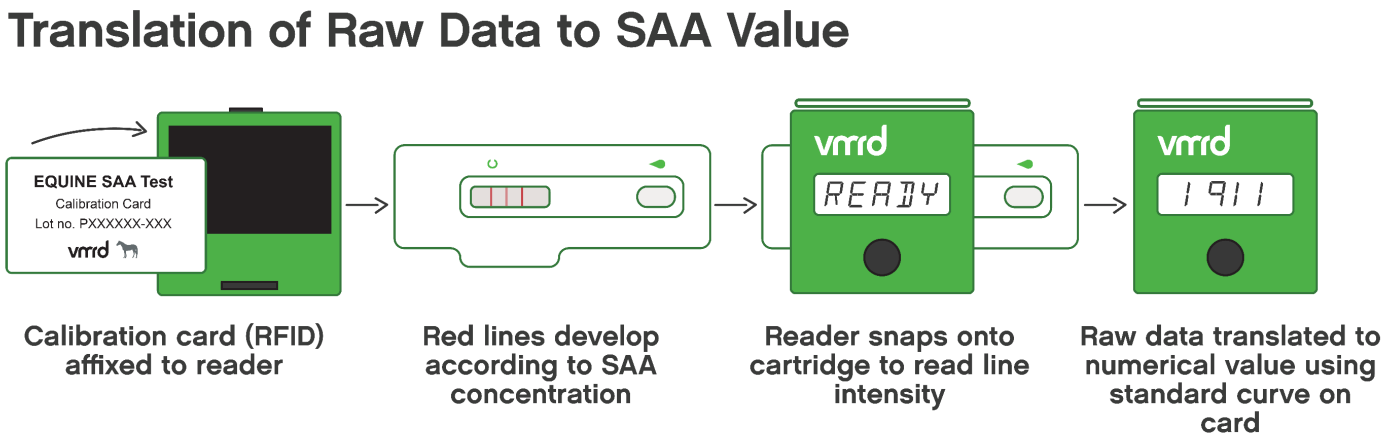
To calibrate VMRD SAA, we use clinical samples from real animals as standards, with the same samples tested in each lot to ensure consistency. The SAA values of these samples were established by thorough testing with Eiken LZ-SAA, and they were then stored at -80°C in single-use aliquots for long-term use. During manufacturing, these standards are used as reference points to ensure that each lot/batch of tests is performing as expected and generating consistent results from one lot to the next.

How does the calibration card work?

When each lot of tests is completed, we use the results from the calibration samples to create a standard curve, which plots the known SAA concentration for each sample against the raw data obtained when the reader reads the intensity of the red lines on the test cartridge. This lot-specific and test-specific standard curve is programmed onto the calibration card, which communicates the information to the VMRD reader via RFID technology when the card is attached to the reader.



When a patient sample is run, the red lines on the test develop according to SAA concentration. The reader measures the intensity of the 3 lines (2 test lines and 1 control) and uses the standard curve from the calibration card to translate those raw numbers into an SAA value. In this manner, the reader is able to adjust interpretation of the test cartridge based on specifications for that individual lot and test type.



All diagnostic assays have some degree of unavoidable variability between lots that occurs during manufacturing. However, this process of lot-specific calibration using a standard curve mitigates any existing variability to provide consistency and reliability over time and between readers.

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