

The Calibration of VMRD Serum Amyloid A (SAA)

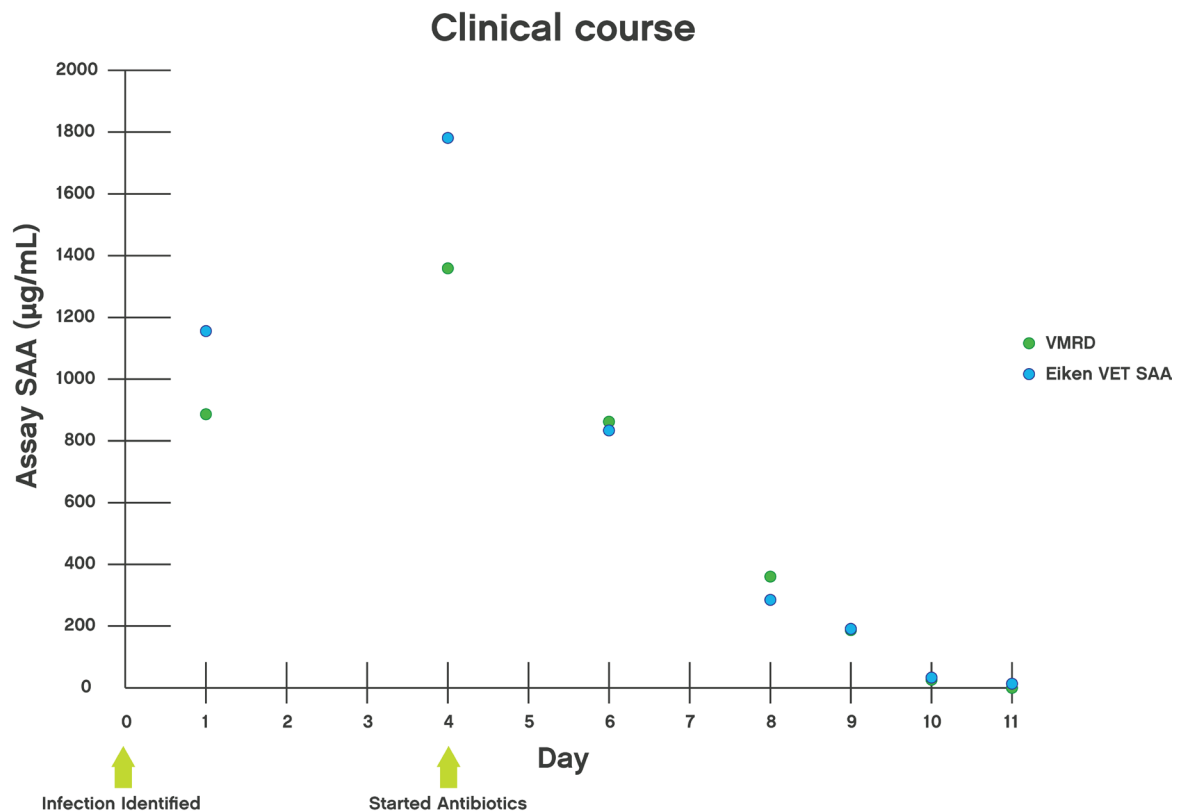
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One of the key advantages for using VMRD's SAA test for equine, canine, and feline is the accuracy and consistency provided by the calibration system.

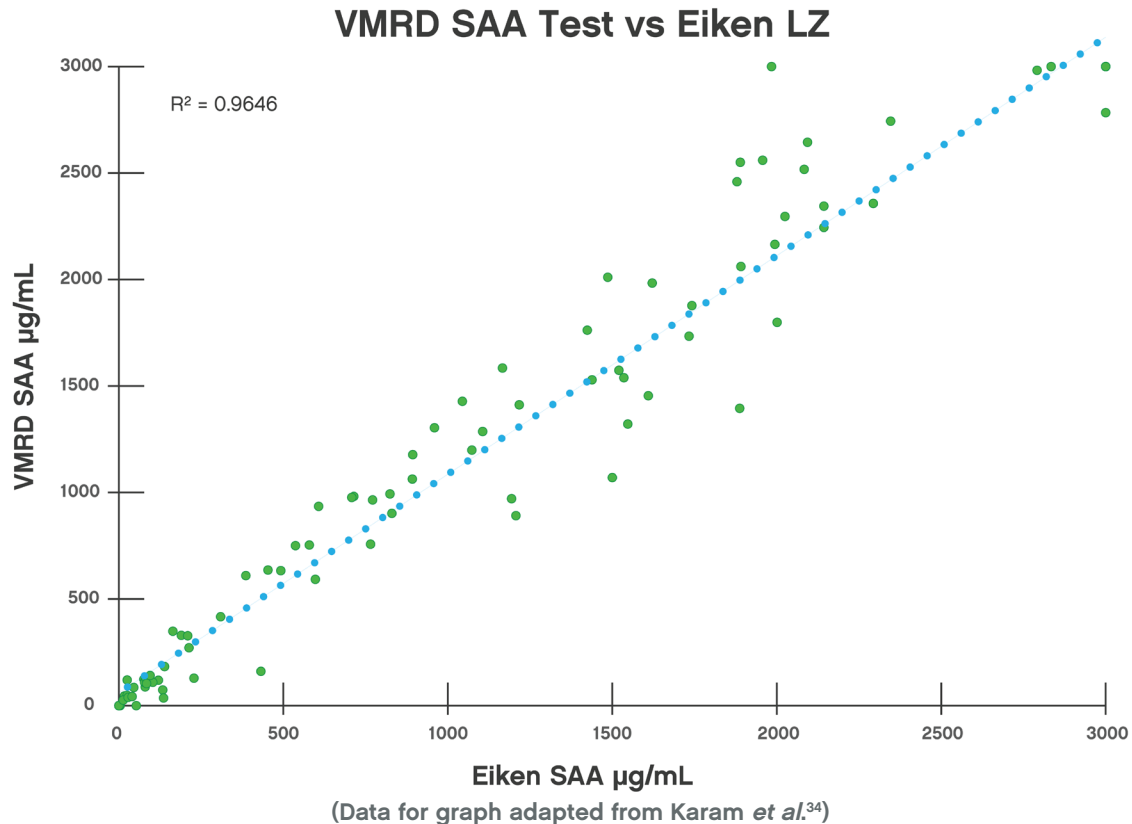
SAA is an "intrinsically disordered" protein.

SAA exists in varying configurations and changes structure frequently, including binding with cholesterol in the bloodstream, making absolute quantification a challenge. Different assays have different components that may bind differently to the variable structures of SAA, therefore it is not unexpected that absolute numbers may vary between assays.¹ This is very similar to how reference ranges for hematology and 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.



Eiken LZ-SAA was the original reference standard for 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 publications³⁻³¹, and specifically validated for equine³² and feline³³ samples. In the independent validation study performed by Dr. Nicola Pusterla at UC Davis, the equine VMRD SAA assay correlated very well with Eiken LZ-SAA.³⁴



VMRD SAA is now calibrated against Eiken VET-SAA

In 2022, 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 reference 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, and reference labs will only report values up to 3000 µg/mL. To align with these changes and ensure results from the VMRD SAA test match well with reference lab testing, we elected to re-calibrate our test to Eiken VET-SAA in late 2022. This was made possible by our lot-specific calibration card system which allows us to be adaptable and keep our VMRD POC products updated to current standards.

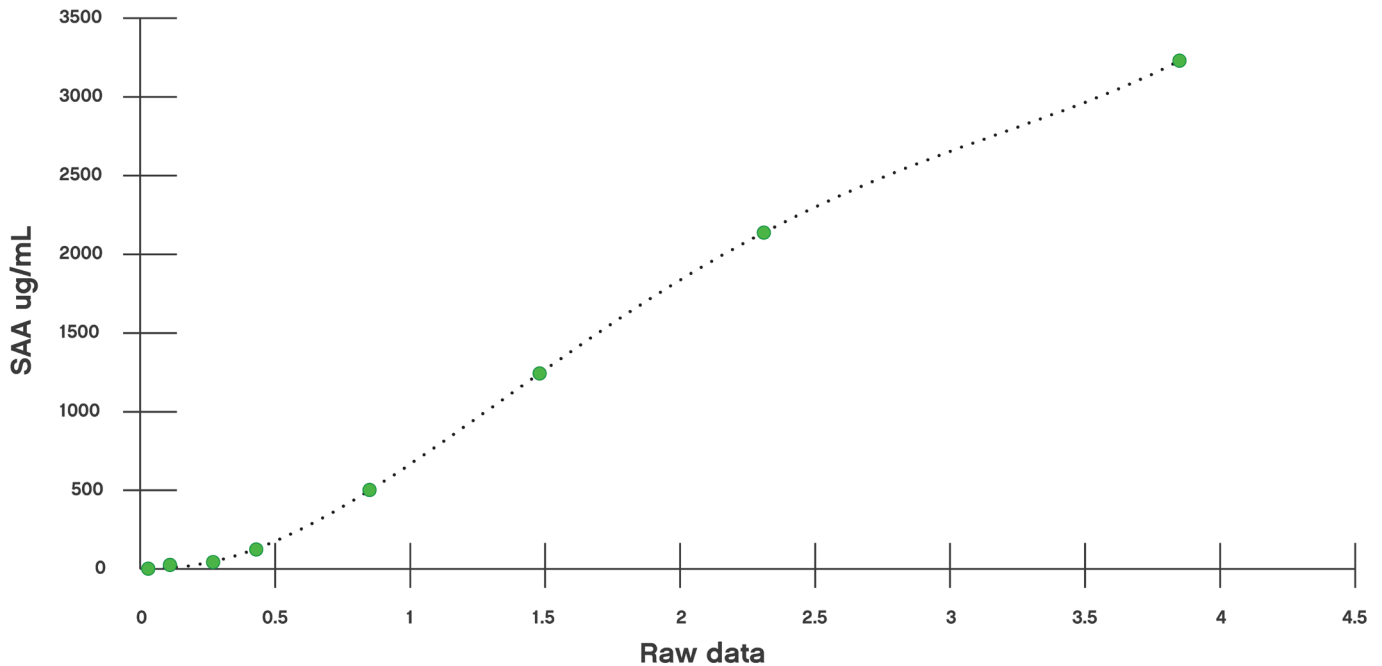
Every batch of VMRD SAA is calibrated for greatest consistency

To calibrate VMRD SAA, we use clinical equine samples as standards, with the same samples tested in each lot to ensure consistency. These samples were tested with Eiken VET-SAA at both Cornell and the University of Miami, 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?

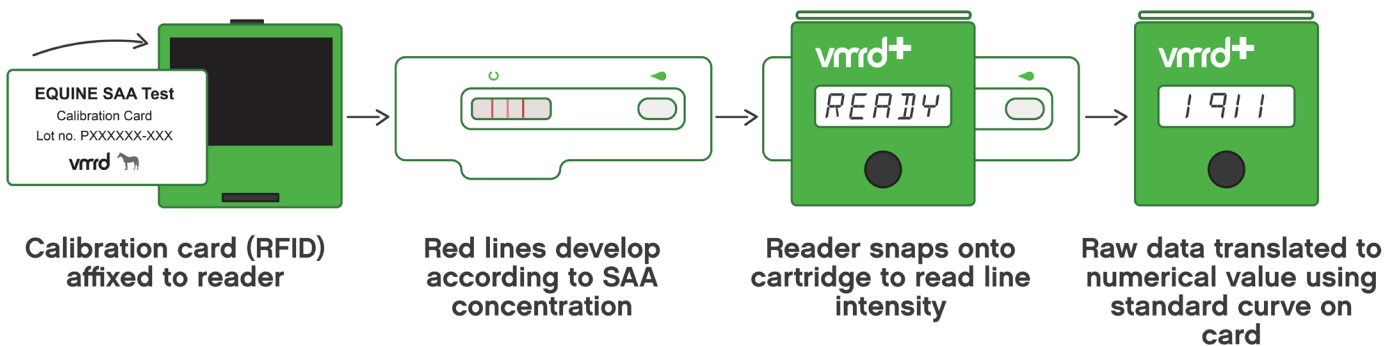
When each lot of tests is completed, we use the results from the calibration samples to create a standard curve, which plots the known SAA concentration for each sample against the raw data obtained when the reader reads the intensity of the red lines on the test cartridge. This lot-specific and test-specific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

Example standard curve



When a patient sample is run, the 2 test lines get darker and the 1 control line ("C") gets lighter as SAA concentration increases. The reader measures the intensity of the 3 lines 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.

Translation of Raw Data to SAA Value



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 maximize consistency and reliability over time and between readers.

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