



Serum Amyloid A (SAA) Test

User Guide

Components



Reader sold separately.

| Component | Quantity |
|-----------|----------|
|-----------|----------|

Each individually packaged test contains:

| | |
|----------------|---|
| Test Cartridge | 1 |
|----------------|---|

| | |
|----------------|---|
| Dilution Tubes | 1 |
|----------------|---|

| | |
|------------------|---|
| Sampling Devices | 1 |
|------------------|---|

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1 The basics

1-1 Testing Serum Amyloid A

Early identification of infection or systemic inflammation is challenging, and initial clinical signs are often subtle. Serum amyloid A (SAA) is a valuable tool as it is rapidly responsive to clinical changes. Virtually undetectable in normal animals it increases within 6-12 hours in acute inflammation and peaks at 1000-fold baseline values – far quicker and more dramatic than fibrinogen or WBC count. As inflammation improves, it begins dropping within 12-24 hours.

This dynamic nature of SAA makes it an ideal marker for evaluating disease severity and monitoring progression or resolution of illness, including response to treatment.

1-2 Quick test facts

10 minute test



Whole blood

- Standard kit can be used with fresh blood, EDTA blood, or heparin blood
- Samples are collected directly from syringe or blood tube



Numerical results

- Objective interpretation & quantification of results with the VMRD Reader



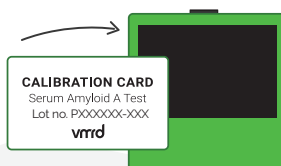
Reader Calibration

- Calibration card keeps results consistent and accurate between readers and over time.
- Results shown in $\mu\text{g/mL}$

To test serum or plasma, contact VMRD to purchase the add-on SAA Serum/Plasma Measurement pack that includes the required sample dilution devices.

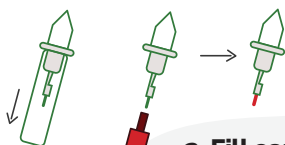
2 Running a test

2-1 Reader-timed **standard test**



1. Attach SAA calibration card

Ensure lot-specific SAA calibration card is attached to the reader



2. Fill capillary

Remove protective cover from capillary and place tip into blood at an angle. Allow to fill, which measures the blood volume needed (5 μL).

Avoid introducing bubbles.

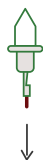
If needed, wipe excess blood from outside of capillary without touching end



3. Puncture foil

Use conical cap to fully puncture foil seal of dilution tube.

Do not use capillary tip.



4. Firmly seat dropper top

Assemble dropper by firmly inserting base of dropper top into mouth of dilution tube.

Ensure dropper top is seated tightly in dilution tube.



*Always use
labeled
Change*



5. Shake horizontally

Vigorously shake dropper **horizontally** for **3-5 seconds** to thoroughly mix blood with diluent.

Results will be shown
after 10 minutes

9. Add 3 drops

Short-press button (<1 sec) for a reader-timed test (10 min)

Button should not be pressed for >1 sec unless an instant read of a completed test is desired (see next page).

Reader will shut off automatically after 50 sec of inactivity

calibration card
"Serum Amyloid A Test"
card when opening a
new box of tests

6. Snap reader on top of cartridge

Press button to turn on (shows **LAST** result)

Press button again for **READY**

8. Discard initial drops

7. Unscrew cap from dropper

2-2 User-timed instant read

- For use **ONLY** with cartridges that will be run outside of reader and **timed by the user**.
- Often used so that multiple tests can be run simultaneously and read using the same reader. It is recommended to wait about 30-60 seconds between starting each test to allow enough time to read at completion.
- Each test must be timed and the result **read at 10 minutes**.

Running the test

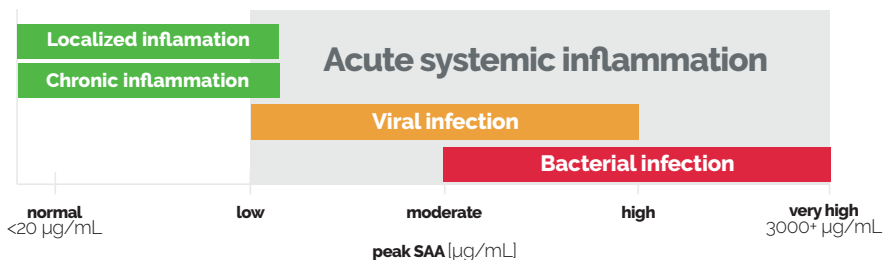
- To prepare samples, follow steps 1 through 5 in section 2-1.
- Unscrew conical cap from dropper and discard initial 3 drops.
- Hold dropper vertically and add 3 drops of diluted sample to fill sample well. Avoid touching dropper to cartridge or dispensed liquid.
- Start your timer (10 min) immediately after applying sample.

Reading the test

- When approximately 15 seconds remains on the timer, press button to turn reader on (previous test result will be shown).
- Press button again to move to **READY** screen.
- When 10 minutes has elapsed, fit reader over cartridge test window and **long-press button (>1 sec)** to perform an instant read. The reader will display **SCAN** while reading calibration card, and then show result ($\mu\text{g/mL}$).

3 Interpretation of results

- **0 to 20 $\mu\text{g/mL}$** - Normal SAA
- **20 to 100 $\mu\text{g/mL}$** - Likely chronic or local process; possible developing or resolving systemic issue
- **100+ $\mu\text{g/mL}$** - indicates systemic inflammation, likely infection



- Visual reading can only be used to estimate concentration of SAA
- Presence of control line (C) indicates successful development of test
- Two SAA test lines increase in intensity with increased sample SAA
- Control line (C) will decrease in intensity with high quantities of SAA

4 Precautions and limitations

Disclaimer: This product is intended to measure the amount of Serum Amyloid A (SAA) in equine blood. It does not diagnose any specific disease or injury and should only be used by or under the supervision of a licensed veterinarian as a tool to identify the presence and degree of systemic inflammation.

- Sample handling and quality may impact test performance.
- Always check for development of control line if there is concern regarding test performance. Control line will become less dark as SAA in sample increases, but should always be present



No food or drink

Do not eat, drink, or smoke while using this product.



Ambient temperature

Avoid running in extreme temperatures when possible, as this may affect the reported result

5 Reader technical information

Calibration card

- **If you use your VMRD reader for both SAA and IgG testing**, make sure you are using the correct card for the type of test you are currently running.
- Each batch of tests is assigned a lot number during manufacturing. The associated calibration card is embedded with the optimal settings for that specific lot number and type of test. This eliminates variability to maximize accuracy across lots, providing you with consistent results over time.
- The calibration card provides the programming needed to run the test which is transmitted to the reader via RFID technology. Therefore, the **calibration card must be attached to the reader for use.**
- The calibration card should be **changed every time a new box of tests is opened.** The new calibration card can be found in the lid of the green test box. Alternatively, you can compare the lot number of the new box with the lot number printed on the current card. If they match, the card does not need to be changed.

Reader operation & screen text

- When the button is **first** pressed, the last test result will be displayed, **LAST** and value in µg/mL.
- Pressing the button a **second** time will bring you to the **READY** screen, at which time the reader is ready for the diluted sample to be applied if running a standard test.
- After applying sample, the button must be pressed a **third** time to start the test. The reader will display **SCAN** as it scans for the calibration card.
- When reading the result, the reader will display **RUN**.
- The reader will **turn off automatically after 50 seconds of inactivity.** There is no manual "off" function.

Batteries & maintenance







- Reader batteries are included in separate packaging in the travel box, labeled "Batteries Inside". Insert before use.
- Takes **3 CR 2032 batteries**, available at online retailers and most stores. Purchased battery quality may vary. It is recommended to use battery equivalents to Energizer or Duracell if available, or VARTA if outside of the U.S.

- Typical **battery life is 100+ tests.**
- The battery compartment is located on the side of the reader and can be opened using a flat object such as a coin. Please contact VMRD if a replacement battery cover is needed.
- Battery performance may be temporarily affected when used at very low temperatures or when running numerous consecutive tests. **Battery life will gradually restore** once the reader is warmed or left unused for a period of time.
- If the battery indicator has displayed consistently for multiple tests, batteries should be changed immediately.
- Batteries can drain over time when left inside the reader during periods of disuse.
- Avoid exposure to water.
- Clean reading window by wiping with water or alcohol if smudged or dirty.

Stored data

- Result of previous test will be stored and viewable on the reader screen until a new test is started. These results will then be transferred to long-term storage.
- Up to **100 results will be stored** on the reader and can be downloaded to a computer via USB cable. Data can then be transferred into Excel in tab delimited (tsv) format. If you are interested in this feature, please contact VMRD to obtain the USB cable and DataReader software.

Error messages

-  Sensor exposed to direct light at start-up: Let the reader turn off (automatic after 50 sec. of inactivity) then turn it on again, ensuring underside of reader is protected from bright light.
-  Battery too low to complete test: Change batteries, or, if due to low ambient temperature, allow reader to warm up which should restore battery life.
- The reader flashes  and  then shows  after pressing the button to start the test: This indicates the calibration card is not properly attached. See Section 6 Troubleshooting, "My reader won't progress past the  screen."
- For any other errors, please contact VMRD Technical Support at support@vmrd.com.

6 Troubleshooting

I got a negative result in a horse that is clearly sick – is your test wrong?

- This is most likely due to the nature of the illness rather than test failure. For example, localized infections or mild inflammation will not cause a significant systemic inflammatory response, which is the driving force behind elevation in SAA. Likewise, allergic responses do not induce the type of inflammatory pathways that stimulate production of SAA. Additionally, although **SAA increases very rapidly** following an inflammatory insult (within 12 hours), it is possible for a horse to show clinical signs of acute illness before measurable levels of SAA have been produced.

I ran your test and a different SAA test at the same time and got different results. Which one is right?

- Slightly different results are expected when using two different types of tests and cannot be directly compared.
- The VMRD SAA test is **calibrated** against the **reference standard** Eiken LZ-SAA test and is kept up-to-date through use of our lot-specific calibration cards, which ensure the most accurate and consistent results.

I forgot my reader or the battery died and I need to run a test. Can I interpret the test visually?

- **Yes**, if a reader is not available the intensity of the test lines can be evaluated visually after letting the cartridge develop for 10 minutes. Increasing intensity of the first two lines (test lines) indicates increasing SAA concentration. Very faint lines may be visible in negative sample. The third line is the control line (C) and should develop whether SAA is present in the sample or not. It will decrease in intensity with very high levels of SAA but should still be present in a test that has run properly (see page 5).

The following situations may affect results to varying degrees:

Minimal effect:

- Extra blood on the outside of the sampling device.
- Small bubble in the capillary tube.
- Too many drops in the sample well.

Significant effect:

- Too few (<3) drops applied — test and control lines may not develop appropriately
- Sample is not thoroughly mixed with diluent.

I used the wrong calibration card. How will this affect my results?

- If the card is **not labeled "Serum Amyloid A Test"** (i.e. is intended for use with a different test), **the results are not valid.**
- Use of the **proper lot-specific calibration card** (matching lot numbers between test and card) ensures maximal accuracy, as there is always some variation between batches/lots for any diagnostic test.
- If the lot number of the test you ran is the same as the lot number on the card, there will be no effect on the results.
- If the calibration card is from a different lot of VMRD SAA, the result should be clinically accurate but should not be used for comparison to other results for monitoring a specific patient. There may also be an increased likelihood of false negative results from low positive samples or very low positive results from negative samples.

My reader won't progress past the READY screen, and flashes SCAN if I try to run a test. What is going on?

- Check to make sure the calibration card is **properly attached** to the reader (velcroed firmly, with top of card flush with top of reader)
- In rare circumstances, the calibration card may have been compromised. Please contact VMRD if you continue to encounter this issue despite proper attachment of the calibration card.

I'm trying to run a timed test and the reader gives me a value when I press the button at the "READY" screen. What is going on?

- Make sure you are pressing the button for the appropriate duration of time.
- A **short press (<1 sec)** at the **READY** screen will start the 10-minute timed option for the test, with the test read occurring after 10 minutes have elapsed.
- If the button is pressed for **more than 1 second** at this point, the reader will read the cartridge immediately and give a value. This is designed to be used on a test that has already been run and independently timed for 10 minutes prior to reading.

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Contact us at +1.509.334.5815 • vets@vmrd.com
VMRD, Inc. Pullman, WA 99163, USA

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