



Serum Amyloid A Test

User Guide

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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 horses, 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



Whole blood

- Can be used with fresh blood, EDTA blood, or heparin blood
- Samples are collected directly from syringe or from blood tube (test within 12 hours of collection)



Numerical results

- Objective interpretation & quantification of results with VMRD Reader
- Results range from <math><20 \mu\text{g/mL}</math> (normal) up to $>3000 \mu\text{g/mL}$



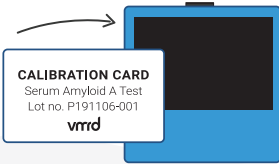
Reader calibration

- Calibration card keeps results consistent and accurate between readers and over time.

10-minute test

2 Running a test

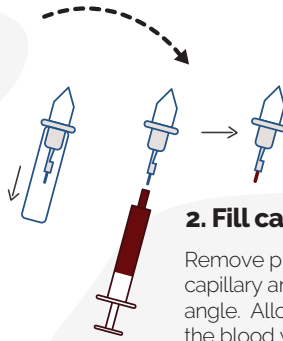
2-1 Standard test in reader



1. Attach calibration card

Attach lot-specific calibration card to the reader

Always change calibration card when using a new box of tests



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**).

Ensure capillary fills completely. 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.

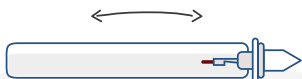
Do not use capillary tip.



4. Firmly seat dropper top

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





5. Shake horizontally

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



6. Snap reader on top of cartridge

- Press button to turn on (shows **LAST** result)
- Press button again for **READY**



7. Unscrew cap

from dropper

8. Discard

initial drops



9. Add 3 drops

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

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

Reader will shut off automatically after 50 sec of inactivity

2-2 Testing multiple samples

- For use ONLY with cartridges that will be run outside of reader using independent timers. In this manner, 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 result read at 10 minutes.

Running test

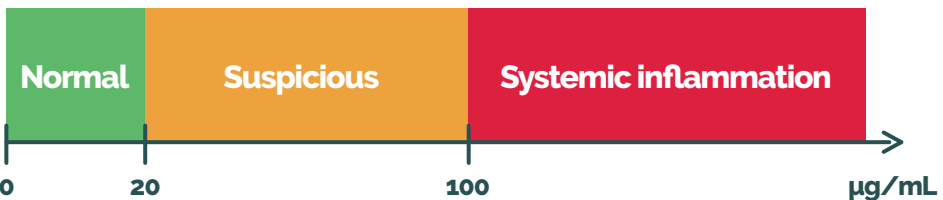
- To prepare samples, follow steps 1 through 5 in section 2-1.
- Unscrew conical cap from dropper and discard initial 3 drops.
- Hold dropper vertically and add 3 drops of diluted sample to fill sample well. Avoid touching dropper to cartridge or dispensed liquid.
- Start your timer (10 min) immediately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

Reading test

- Just before test is ready to be read, press button to turn reader on (previous test result will be shown).
- Press button again to move to **READY** screen.
- After 10 minutes, fit reader over cartridge test window and long-press button >1 second 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 $\mu\text{g/mL}$** - no systemic inflammation (normal SAA)
- **20 to 100 $\mu\text{g/mL}$** - may indicate primarily local process; possible developing or resolving systemic inflammation
- **100+ $\mu\text{g/mL}$** - indicates likely systemic inflammation and possible infection



4 Reader technical information

Calibration card

- Use of the proper lot-specific calibration card **provides your reader with the optimal settings** for the lot of tests being used. You can ensure that you are using the appropriate card by verifying that the lot number printed on the calibration card matches the lot number printed on the test. Calibrating the reader corrects for any variation between test lots and ensures that the reader delivers the most **accurate and consistent results**.
- The calibration card provides the programming needed for the reader to run the test; 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 $\mu\text{g/mL}$.
- 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 reader-timed 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 cartridge, the reader will display **RUN**.
- The reader will turn off automatically after 50 seconds of inactivity. There is no manual "off" function.

Batteries & maintenance

- Clean reading window with water or alcohol if smudged or dirty.
- Takes **3 CR 2032 batteries**, available at online retailers and most stores.
- 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.
- When the battery begins to run low, it will provide **ample warning, enabling you to run** several additional tests until it is convenient to change batteries.

- 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.

Stored data

- Results 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 (csv) format. If you are interested in this feature, please contact VMRD to obtain the USB cable and DataReader software.

Error messages

- Error messages may occur if calibration card is not attached or if reader window is exposed to in bright, direct light.
- If any other errors occur, please contact VMRD for technical support.

5 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.



No food, 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

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 **gold standard** Eiken 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 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.

The following situations should not have a significant clinical effect on your results, but may affect numerical results slightly:

- Extra blood on the outside of the sampling device.
- Bubble in the capillary tube.
- Too many drops in the sample well.
- If <3 drops are applied, the test and control lines may not develop appropriately – it is better to have too much of the diluted sample applied than not enough.

I did not change my calibration card.

How will this affect my results?

- 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 than the test, the result will be clinically accurate but should not be used for comparison to other results for monitoring a specific patient. There may also be a increased likelihood of false negative results from low positive samples or false 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)
- If still having issues, calibration card may have been compromised.
- If this is not the first time using reader, pressing the button again (<1 sec) while reader is displaying **SCAN** will allow you to bypass the calibration card scan and default to the last calibration used by the reader. While this may not provide the highest accuracy, it will allow you to run a test even if the current card is not functioning properly and should provide clinically relevant results.

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?

- 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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Version 2

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