



## False Positive and False Negative Results in Heartworm Disease Testing

Heartworm antigen testing is perceived to be very accurate and precise, with specificity and sensitivity values for most tests reported to be >95%, and with specificity and sensitivity data approaching 100% when three or more adult worms are present. Note that published specificity and sensitivity values are never exactly 100% and those reported values do not account for human error or sample related issues. Therefore, there is no perfect heartworm test in the real world and false positives and negatives do occur.

It is important to understand the true definitions of the following terms prior to discussing details and meaning of test results.

**Specificity:** This term is often perceived to mean accuracy, however, specificity is by definition the measurement of the frequency with which a result will be negative (or normal) in patients without the disease. To clarify, a test with 98% specificity would mean that if 100 negative or normal patients are tested, then the test would in fact show negative or normal in 98 of them, while 2 of the patients would show a false positive. The formula for specificity is:

$$^1\text{Specificity (\%)} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}} \times 100$$

**Sensitivity:** This term indicates the frequency of a positive (or abnormal) test in patients that have the disease. To clarify, for a test that has 98% sensitivity and 100 known positive patients are tested, then 2 of the patients will have a false negative result. The formula for sensitivity is:

$$^1\text{Sensitivity (\%)} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \times 100$$

The truth about specificity and sensitivity is that they are determined using animals or samples of known disease status, compared against a “gold standard” or highly accepted reference test. In heartworm disease testing, these values are generated using samples from deceased animals that have known numbers of heartworms based on necropsy. In the real world, however, because the veterinarian does not know the true disease status of the patients they are evaluating, the values for sensitivity and specificity are by themselves far less relevant.

Veterinarians investigate disease through history, clinical signs, physical examination findings as well as other testing modalities. Patients are also presented for routine wellness examinations which often include screening tests when there is no history of illness or abnormal clinical signs (wellness examinations). It is vital that the veterinarian understand the true positive and negative predictive value of the screening tests they are using to perform the highest quality diagnostic medicine.

Predictive values are determined using specificity and sensitivity data, as well as incorporating the likelihood that the disease will occur based on its prevalence in the local population, as well as patient history, clinical signs and other test results. The difficulty occurs when the test is being used as a screening tool for a disease such as heartworm where the patient’s owner claims to be giving preventative on the prescribed basis and there are no clinical signs -- in that case, as veterinarians, all we have to go on is the result of the test.





**Positive Predictive Value:** This is the percentage of patients with a positive test that actually have the disease in question (True Positives) and includes either prevalence data or likelihood based on illness. **Negative Predictive Value** is the percentage of patients who test negative to a disease and are in fact negative (True Negatives), but also accounting for prevalence data or clinical illness. **Prevalence** is the percentage of true positive animals within the tested population. The formula for both of these are quite complex (incorporating specificity, sensitivity and prevalence data), but when interpreting the diagnostic result, it is imperative to include either prevalence data for a screening protocol, or history and clinical signs in an ill patient.

Example 1: A patient in California visiting the veterinarian for a routine wellness visit tests positive on an in-house test for heartworm disease. The owner has great compliance and says they give preventative every month as directed. The in-house test used has a sensitivity and specificity of 98% and the prevalence data for the area indicates 1.6% of the dogs actually have heartworm disease (True Positives). Taking into account these values, the following values are calculated:

Positive Predictive Value = 61.68%

Negative Predictive Value = 99.98%

As you can see, 1 out of 3 times in this example, even with 98% specificity and sensitivity, a patient testing positive on routine screening will actually be a False Positive (even with specificity and sensitivity values of 98%). However, a patient testing negative is almost assuredly a True Negative.

Example 2: A patient in California visits the veterinarian for weight loss and coughing. The CBC indicates an eosinophilia and there is mild liver enzyme elevation. Thoracic radiographs indicate tortuous blood vessels in the lungs. In this case, the veterinarian believes that heartworm disease is likely, feeling about 90% sure. The heartworm antigen test is positive. If we use the 90% value as our prevalence figure, given the clinical signs and supporting data, we calculate the following values:

Positive Predictive Value = 99.89%

Negative Predictive Value = 91.67%

In this case, we should actually question the negative test if it had occurred, but the positive test is virtually a certainty.

Below is a chart with positive and negative predictive values based on prevalence data of heartworm disease in a paper by *Bowman et al*<sup>2</sup> and using specificity and sensitivity values of 98%. This chart would be valid for patients without clinical signs or history that would indicate disease.

Prevalence	Positive Predictive Value	Negative Predictive Value
0.5	33.22%	99.99%
1.0	50.00%	99.99%
1.5	60.12%	99.98%
2.0	66.89%	99.98%
2.5	71.74%	99.97%
3.0	75.38%	99.97%
4.0	80.49%	99.96%
5.0	83.90%	99.95%
6.0	86.34%	99.94%
7.0	88.17%	99.92%

As a point of reference, the state with the lowest prevalence was South Dakota at 0.1% and the highest was Mississippi at 7.4%<sup>2</sup>.





**False Positive Tests:** The question now is this: what do we do with positive tests, especially in patients presented for routine health checks and without history or clinical signs consistent with heartworm disease?

The American Heartworm Society, in its 2010 Canine Guidelines (<http://www.heartwormsociety.org/veterinary-resources/canine-guidelines.html#3>) states: "If the validity of a weakly positive result is in doubt, verification may be achieved by repeating the test and if still ambiguous, independent confirmation by some other means, such as a different antigen test format." Therefore, if a positive test occurs where there is no supportive history or clinical signs consistent with the disease, the test should be either repeated or confirmed with an antigen test of a different type. They also state: "Concentration tests for microfilariae, thoracic radiography to detect signs of heartworm disease or ultrasonographic visualization of worms may also validate weakly positive antigen test results."

In summary, for positive tests, it is recommended that the positive test be verified by either an antigen test of some other methodology than that used in the original test, or by additional supportive diagnostics for the disease. Also, testing for circulating microfilaria, Knott's testing or direct blood smear, is recommended.

**False Negative Tests:** The American Heartworm Society states: "False negative results also can occur rarely with any one test, so unexpected negative results should be followed by retesting with a different test." Therefore, if heartworm disease is suspected and a negative test occurs, a second test of differing methodology should be used to retest the patient.

Abaxis offers two antigen tests with different methodologies. The individual Canine Heartworm Antigen Test is a lateral flow sandwich assay. The test provided on the Canine Wellness Panel is a particle-assisted sol-particle aggregation immunoassay. The Abaxis customer, therefore, can easily confirm both unexpected positive as well as unexpected negative heartworm test results in the office and still be able to report results and discuss treatment options and prognosis with the client in the office.

Also, keep in mind that false positive tests occur just as frequently at commercial labs as occurs in-house. When a positive test occurs at the commercial lab, it is retested immediately. If the second test is negative and is then confirmed negative, the only thing the veterinarian sees on the report is the negative results -- they are never alerted to the false positive. Similarly, if a positive does occur, the report will read "rechecked and verified" or "confirmed by repeat analysis". This is a clear indication that at least one confirmatory test has been performed to confirm the original.

1. Thrall, M.A.(ed). *Veterinary Hematological and Clinical Chemistry*. Blackwell Publishing, Ames, Iowa, pp. 50-51.
2. Bowman, D., Little, S.E., Lorentzen, L., Shields, J., Sullivan, M.P., Carlin, E.P. 2009. Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Veterinary Parasitology* 2009;160:138-148

