



# Diagnostic testing in the shelter environment

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## What is a diagnostic test?

Any procedure used to determine whether a disease is present can be considered a diagnostic test. Some “tests” commonly employed in shelters include:

1. Observation of physical signs and symptoms
2. Fecal float
3. Skin scraping
4. Woods lamp examination for ringworm
5. Fungal culture
6. Blood or fecal tests for FeLV, FIV, parvo, heartworm, others
7. Temperament tests/behavior assessment

## Why test?

Diagnostic testing in shelters may serve multiple purposes, some in common with private practice and some unique to shelters, including:

1. Population protection
  - a. Identification and segregation or removal of infectious animals
  - b. Periodic surveillance: identification of prevailing causes of common syndromes to evaluate management and treatment strategies
2. Human protection (adopters, shelter staff and volunteers)
  - a. Recognition of zoonotic conditions
  - b. Recognition of serious and/or expensive conditions that could lead to heartbreak
  - c. Recognition of dangerous or problematic behaviors
3. Individual animal protection
  - a. Early recognition and treatment of disease
4. Risk assessment prior to investment
  - a. for example, feline retroviral testing prior to sending to foster care or treating for other conditions

## High stakes: why worry about diagnostic tests?

All diagnostic tests lead to occasional inaccurate results. As is often the case in shelters, the stakes in diagnostic testing are high either way: false negative test results can lead to a false sense of security and devastating outbreaks, while a false positive can needlessly cost an animal its life. It is crucial that tests be used in a way that maximizes the chance

that an accurate result will be obtained. A well-thought out diagnostic testing strategy is a key component of a preventive medicine plan in a shelter.

## What questions need to be asked before deciding on a diagnostic testing strategy?

No single recipe for diagnostic testing is appropriate for all shelters. Tests must be chosen, administered and interpreted in a way that maximizes the benefit while minimizing the cost, both for the population and individual animals. Factors to consider in developing a testing program include:

1. Whether to test at all
2. How accurate is the test, and what factors can influence accuracy
3. Which tests to use
4. Which animals to test
  - a. All animals at risk (screening test)
  - b. Certain asymptomatic animals at increased risk
  - c. Only symptomatic animals
5. What actions will be taken based on test results.
  - a. Confirmatory testing
  - b. Isolation
  - c. Treatment
  - d. Do not admit to shelter
  - e. Euthanasia

## To test or not to test?

The answer to this question is not always immediately obvious. Testing should only be performed when the benefits of doing so outweigh the risks. In addition to the cost of the test itself, the accuracy of available tests must be considered. The risks of not testing (or accepting a false negative result) include:

1. Failure to detect an infectious disease that can spread within the population and/or cause zoonotic disease.
2. Failure to identify a condition requiring treatment.
3. Failure to identify a sick animal that then gets adopted out, causing heartbreak, angry phone calls, lawsuits, etc.



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For a disease where these risks are high, testing is a high priority, and a test that is unlikely to give false negative results is preferred (high negative predictive value). *Serious infectious and zoonotic conditions fall into this category, as are conditions where early intervention is key to a good prognosis.*

The risk of testing (or accepting a false positive result) includes:

1. Euthanasia or rejection of an animal that is not really ill (including stigma of disease that may discourage adopters.)
2. Cost and possible complications of treatment.
3. Cost and staff time required for testing takes resources away from other needed programs.

For a disease that poses a low population risk, the risk of testing at all may outweigh the risk of not testing, particularly if the disease in question is uncommon or if the only available test is error prone and/or expensive. If the decision is made to test, a test that is unlikely to give false positives is preferred.

## What determines test accuracy?

Whenever testing is performed, inaccurate results will occur some percentage of the time. Some cats test positive for FeLV even though they aren't really infected, some dogs pass a temperament test with flying colors and then go on to bite someone, etc. False results occur due to characteristics of the test itself, frequency of the condition in the tested population, an assortment of biological factors, and of course mishandling of the test or sample.

## First, some terminology:

A test may result in one of four outcomes: true positive, true negative, false positive or false negative. Some terms used to describe the likelihood of various test outcomes are as follows:

**Sensitivity:** The probability that an animal who does have the disease in question will test positive for the disease. A very sensitive test recognizes almost all animals that have the disease as positive, and leads to few false negatives.

**Specificity:** The probability that an animal who does not have the disease in question will test negative. A very specific test means very few animals that don't have the disease will test positive (few false positives).

**Positive predictive value (PPV):** The probability that a positive test result is true (the animal actually has the disease in question). PPV is high when a test is very specific (few false positives) and the disease is common in the population being tested.

$$PPV = (\text{true test positives} / \text{total test positives})$$

**Negative predictive value (NPV):** The probability that a negative test result is true (the animal really doesn't have the disease in question). NPV is high when a test is very sensitive (few false negatives) and the disease is rare in the population being tested.

$$NPV = (\text{true test negatives} / \text{total test negatives})$$

## Evaluating sensitivity and specificity

An ideal test has both high specificity and high sensitivity, leading to accurate prediction of true disease status. Often, however, a trade-off is required. A very sensitive test is frequently less specific (results in more false positives) and vice versa. Some of the tests commonly used in shelters have been evaluated by independent studies for sensitivity and specificity (references 2-11). Experts in the field may be able to give an idea of test accuracy, even if independent studies can't be found for that test. Estimates for some commonly used tests are included in the description of diagnostic testing for specific diseases.

## Choosing a test strategy to improve sensitivity or specificity

Testing strategy and interpretation of results can be used to manipulate sensitivity and specificity to some extent. This is especially helpful when no single test has the desired characteristics.

### *Specificity of testing can be increased by:*

Using 2 tests and requiring that both be positive to call an animal infected. For instance, one could require that a cat test positive on both an ELISA and western blot test for FIV before being considered infected.

### *Sensitivity of testing can be increased by:*

Using 2 tests and calling an animal positive if it is positive on either test. For instance, one could consider a dog parvo positive if it tests positive on a fecal ELISA or if it has symptoms and decreased white blood cells on a blood smear.



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## Evaluating predictive value

Predictive value is ultimately the most important thing to know about a test, since it tells you how much faith you can put in a given test result. Calculation of predictive value requires knowledge of test sensitivity, specificity and disease frequency in the test population.

**Example 1** (*appendix-1*) demonstrates calculation of PPV and NPV for a hypothetical FIV test. In this example, even though the sensitivity and specificity of the test are quite good, in this particular population a positive test result is correct only 15% of the time, although a negative result is correct over 99% of the time. The poor positive predictive value of the test is mainly due to the low frequency of disease in this population. This will be discussed further in the section on disease frequency below.

## Effect of test population and disease frequency on predictive value

No matter how sensitive or specific the test, positive predictive value is decreased when the disease is uncommon in the population being tested, and vice versa. This can be a concern when using a screening test on a healthy population, as was demonstrated in example 1. Sometimes estimates of disease frequency are available in the literature (references 12-18) and true disease prevalence can also be estimated by a simple formula (famous last words!) described in example 3 (*appendix 1*).

Although the numbers given in example 1 are used for demonstration purposes only, the effect of disease frequency on predictive value is not simply a hypothetical concern. For example, one study estimated that as many as 69% of positive results from FeLV ELISA tests in veterinary hospitals were incorrect (reference 6)! This may make the shelter practitioner think twice about the utility of using these tests in shelters. In addition to medical reasons for testing, however, it is important to consider the political and emotional costs of adopting out animals that go on to test positive for serious illness at veterinary clinics soon afterwards.

## Improving predictive value

If the consequences of a false positive test result are very severe (such as euthanasia of the individual), a test with high positive predictive value is desired. If the consequences of a false negative test result are potentially devastating (such as spread of

parvo to the entire shelter population), high negative predictive value is crucial.

Positive predictive value can be improved by selecting a test population in which the disease is more likely, as shown in example 2 (*appendix 1*). This does not mean that testing need actually be restricted only to this population, but simply bear in mind that more confidence should be placed in a positive result in an animal with a consistent background (high risk age, breed, sex, etc.) and clinical signs suggestive of the condition in question.

Positive and negative predictive value can also be manipulated by choosing tests (or test combinations) with greater sensitivity or specificity, as discussed above. This is the best way to improve negative predictive value (applying the test to a population in which the disease is infrequent would also work, but makes little sense). Choosing a more sensitive test or test combination will improve negative predictive value, while choosing a more specific test or requiring more than one test criteria to make the diagnosis will lead to improved positive predictive value.

## Other factors influencing test accuracy: what is the test looking for?

In addition to test sensitivity, specificity and predictive value, biological factors influence whether test results can be accurately interpreted. To understand what can interfere with test interpretation, it is necessary to know what the test is designed to detect. Tests typically “look” for antibodies to disease, for some component of the disease-causing agent (antigen) or the agent itself (fungal culture, fecal float, etc.). FIV and distemper are two common diseases in shelters that can be tested for by looking for antibodies. Parvo, panleukopenia, FeLV and most heartworm snap tests detect antigen.



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*False negative results can occur because the test sample does not contain the antigen or antibody the test looks for, even though the animal is infected. Reasons for this include:*

1. Antibodies not being produced
  - a. Due to age (puppies and kittens), concurrent or overwhelming disease
  - b. Recent infection (not enough time for antibodies to be produced)
2. Antigen no longer being shed late in infection, or not yet being shed early in infection
  - a. E.g. parvo test false negative after day 5-7 of clinical disease (reference 19)
3. Antigen not present in test sample even though animal is systemically infected
  - a. E.g. saliva/tear test for FeLV prone to false negatives (reference 8)
4. Antigen not yet present due to life stage of organism
  - a. E.g. heartworm test detects adult worms which take six months to develop
5. Antigen all bound by antibody or present at too low a level to be detected
  - a. E.g. cats with heartworm often have only 1 or 2 worms, too few to be detected by many tests

*False positive results can occur because the test sample contains the antigen or antibody the test is looking for, but not because the animal is infected. Reasons for this include:*

1. Antibodies due to vaccination
  - a. Both killed and modified live vaccines produce antibodies
  - b. E.g. FIV vaccine leads to false positive on all available FIV tests
2. Antigen shedding following modified live vaccine administration
  - a. E.g. antigen from recent modified live vaccine for parvo or panleukopenia can cause false positive ELISA test
  - b. Usually weak false positive within 1-2 weeks of vaccination

3. Maternal antibody
  - a. E.g. kittens under 6 months of age may test positive for FIV due to presence of maternal antibodies
4. Environmental contamination
  - a. E.g. positive ringworm culture due to coat contamination in uninfected cat

*False positive results can also occur with some tests due to characteristics of the disease itself:*

1. Animal is truly infected but may recover
  - a. E.g. a cat with recent FeLV infection
2. Animal is infected with a benign agent indistinguishable from a disease causing organism
  - a. E.g. blood tests can not distinguish feline coronavirus infection from FIP
3. Animal is truly infected but that organism if not causing the animal's clinical signs (not exactly a false positive)
  - a. E.g. healthy cats can be culture positive for bordetella infection; isolation from a sick cat does not mean it is causing disease

*And finally, false results occur due to incorrect handling of the test or sample:*

1. The most common reason for inaccurate results in many tests!
2. Testing should only be performed by designated, trained staff.
3. All manufacturer instructions should be followed exactly.
4. For laboratory tests, the quality of the lab can affect the reliability of results.



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## Which animals should be tested?

The population of animals to test should be chosen in order to:

1. Increase the likelihood of accurate results
  - a. Consider disease frequency in that population
  - b. Consider whether the test can be accurately interpreted e.g. can't interpret results in puppies for heartworm, kittens for FIV! Use caution in interpreting positive results for parvo, panleukopenia, distemper in recent vaccinates.
2. Minimize the cost of unnecessary testing
  - a. Consider purpose of testing
    - i. If main purpose is adopter protection, test at adoption or after animal has passed all other screening.
    - ii. If main purpose is population protection, test all or test before group housing (depending on contagiousness).

## What do you do once you have the result?

*When interpreting a test result, there are two choices:*

1. The initial result can be taken at face value
2. Further confirmatory testing can be performed (if available)

*The best choice depends on:*

1. Likelihood that the initial result is correct (predictive value)
2. Availability, cost and practicality of confirmatory testing
3. Housing and management considerations while awaiting further results
4. Consequences of accepting an inaccurate result
  - a. For the individual animal
  - b. For the shelter population
  - c. For the humans involved (adopter and shelter workers)

When screening for disease in a healthy population, negative results are generally accepted at face value (this is a pretty safe bet – recall negative predictive value is high when the likelihood of disease is low in the test population). On the other hand, when the consequences of accepting a false negative are severe and the sensitivity of the test is low, confirmation may be indicated, especially in an animal at high risk for disease. For example, a negative result from Woods lamp screening for ringworm might be

acceptable before adding a healthy adult cat to a group housing area, but the same results in a kitten with characteristic signs of ringworm infection must be viewed with suspicion.

In deciding what do with animals testing positive for infectious disease, shelters must also consider the current fate of healthy animals in their shelter and community. For instance, false positive tests for FeLV are common in some populations, as discussed above. Various confirmatory tests are available, involving additional cost and the logistical difficulties of holding the animal while awaiting results. When choosing between euthanizing unconfirmed FeLV positive and FeLV negative cats that are otherwise of similar adoptability, it makes sense to choose the FeLV positive cats (even with the low positive predictive value, FeLV positive cats are significantly more likely to be infected than their negative counterparts). On the other hand, for a shelter that is choosing between euthanizing an unconfirmed FeLV positive cat and not euthanizing any cat at all, investing in additional testing is indicated provided the cat can be safely housed in the interim. The same logic applies to a limited admission shelter in deciding whether to admit or turn away test positive animals without confirmation.

## How should test results be documented?

Significant amounts of time and money are spent on testing in many shelters. Careful documentation ensures that the most information possible can be extracted from all those test results. Obviously, all test results need to be recorded in the animal's medical record, to go home with the new adopter. In addition, a testing log should be kept for all commonly used tests, including:

1. Date
2. Animal ID
3. Signalment (age, sex, breed)
4. Symptoms (if any)
5. Test type
6. Test results
7. Initials of person performing test

Over time, this data collection may reveal information about risk factors for disease, disease frequency in the shelter population and various sub-populations, and can allow more specific targeting of testing or prevention programs.



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## Appendix 1. page 1

**NOTE:** Values for sensitivity, specificity, and frequency used in this and all other examples in this chapter are intended for demonstration purposes only and do not necessarily reflect actual test accuracy or disease frequency.

### Example 1: Sensitivity, specificity, frequency and predictive value.

A hypothetical FIV test with a sensitivity and specificity of 90% and 95% respectively is applied to a test population of 1000 shelter cats of unknown disease status, with an estimated frequency of FIV infection at 1%.

- **Sensitivity** of 90% means of the 10 true positives, 9 will test positive:  $.90 \times 10 = 9$
- **Specificity** of 95% means that 95% of the truly negative cats will test negative:  $.95 \times 990 = 940.5$  (round off to 940 for simplicity).
- **Frequency** of 1% means that out of 1000 cats, 10 will be infected with FIV, and 990 will be free of the disease.

That leaves 990-940=50 of the truly negative cats test positive. This is shown in diagrammatic form below:

	FIV +	FIV -	total
Test +	9	50	59
Test -	1	940	941
Total	10	990	1000

PPV = (true positives/total positives)  
therefore PPV= (9/59) = .15 (15%).

NPV = (true negatives/total negatives)  
therefore NPV= (940/941) = .9989 (99.89 %)

*A positive test only has a 15% chance of being correct!*

### Example 2: Increasing frequency improves positive predictive value.

Returning to the scenario described in example 1, but this time testing only adult, intact male cats, with an estimated disease frequency of 10% (as opposed to 1% in example 1.)

**Frequency** = 10% x 1000 cats = 100 infected with FIV, and 900 free of the disease.

**Sensitivity** = 90% x 100 truly infected = 90 true positives and 100-90 = 10 false negatives.

**Specificity** = 95% x 900 truly uninfected = 855 true negatives, and 900-855 = 45 false positives. This is shown in diagrammatic form below:

	FIV +	FIV -	total
Test +	90	45	135
Test -	10	855	865
Total	100	990	1000

PPV = (true positives/total positives)  
therefore PPV= (90/135) = .666 (66.6%).

NPV = (true negatives/total negatives)  
therefore NPV= (855/865) = .988 (98.8 %).

So by choosing a test population in which disease is more likely, a positive result is over 4 times more likely to be correct compared to the previous example, and negative predictive value is still quite good.



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## Appendix 1. page 2

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### Example 3. Estimating true disease prevalence from apparent prevalence.

It is sometimes necessary to estimate the true frequency of disease in the test population (as opposed to the number of test positives). In order to calculate this for your shelter population, you will need to keep records of test results until you have a reasonable number of samples (more samples needed the less common positive results are) and obtain an estimate of the sensitivity (SE) and specificity (SP) of the test used. The formula to calculate true prevalence (TP), the total actually diseased out of the total population tested, from the apparent prevalence (AP), the total positive results out of the total population tested is:

$$TP = AP + (SP - 1) / (SE + SP - 1)$$

Using the same numbers as in example 1, but assuming this time we don't know the true frequency but are working backwards from the test results:

$$SE = .9 \text{ (90\%)}$$

$$SP = .95 \text{ (95\%)}$$

$$AP = 59/1000 = .059$$

*(total test positives out of total population tested)*

$$\text{Then } TP = .059 + (.95 - 1) / (.9 + .95 - 1) = .01, \text{ or } 1\%$$

TP = 1%(0.10) which was the true frequency we assumed when we set up the exercise – so it worked!

Go ahead...try it on some of the test data you have collected over the last 6 months in your shelter.

