Interpretation of Diagnostic Tests 2

Example of interpretation of diagnostic test results

The diagnostic question is whether a particular dog does have an infection with adult *Dirofilaria immitis*? As a general strategy, the dog should first be evaluated clinically for presence of clinical symptoms of heartworm disease. This is followed by a microfilaria examination. If microfilariae are present, no further test is required. If no microfilariae are detectable and the clinical history is suggestive of heartworm disease, the diagnostic procedure should be followed by a serological test. The following 4 hypothetical case scenarios will demonstrate the decision process evolving from particular findings at different stages of the examination.

As case 1:

5 year old untreated dog has been presented at a Brisbane clinic. The prevalence in the local dog population is 50% which serves as the first estimate of pre-test probability. During the clinical examination, the animal is found to have a history and clinical signs consistent with heartworm disease. As a consequence of this new information, the pre-test probability is revised to 80%. During the examination of the blood smear microfilariae are found.

A differentiation from non-pathogenic filarids D. repens and *Dipetalonema reconditum* is performed on the basis of morphology and staining characteristics with acid phosphatase. If they are found to be *D. immitis*, a diagnosis of dirofilariasis is made and no further testing is required

Case 2 :

Is another 5 year old untreated dog presented at a Brisbane clinic with the same prevalence of 50% in the dog population (=first estimate of pre-test probability). History and clinical signs appear to be consistent with heartworm disease which results in a revised pre-test probability of 80%. On examination of the blood smear no microfilariae are found, and consequentially the pre-test probability is revised to 60%.

Because of the pre-test probability of 60% the dog is likely to have the disease, the diagnostic test is used to “rule-in” the disease.

The serological test result is positive, which increases the probability of heartworm disease to above 95%.

Case 3

in the local dog population (=first estimate of pre-test probability). Because history and clinical signs are consistent with heartworm disease, the clinician revises the pre-test probability to 80%. Examination of the blood smear does not show any evidence of microfilariae. The pre-test probability is therefore revised to 60%. The serological test is applied with the objective in mind to “rulein” disease.

The result from the serology is negative, which magain is a 5 year old untreated dog presented at a Brisbane clinic with 50% prevalence eans that the probability of the dog not having heartworm disease becomes about 80%. To confirm that the dog does not have the disease, a “rule-out” test with high sensitivity should be used to confirm the result.

As case 4:

5 year old untreated dog is presented at a Brisbane clinic because the owner would like to take the dog to New Zealand, and have the clinician provide a certificate that the dog is free from D.immitis infection. As above, the local infection prevalence is about 50% (= first estimate of pre-test probability). No clinical work-up and no blood smear is used as part of the diagnostic process. A serological test is used with the objective to “rule-out” Dirofilaria immitis infection. The clinician has the choice between different test kits. The operating characteristics of the serological tests are presented in Table 4. A comparison of the tests can be based on estimating the predictive values for positive and negative test results.

Product	Sensitivity (in brackets number of diseased dogs in trial)	Specificity (in brackets number of non diseased dogs in trial)
Diro kit Latex (2 trials)	85.3% (34) + 82.9% (76)	95.7% (23) + 100% (30)
Diromail (ELISA)	92.2%(103)	97.4% (78)
Dirocheck (ELISA)	92% (82) + 73%	99% (91) + 94%
Filarocheck (ELISA)	97.3% (149)	98.2% (165
CITE (ELISA)	85% (266)	100% (176)

Probability	Calculation formula
Positive predictive value	$\frac{Sensitivity \times Prevalence}{Sensitivity \times Prevalence + (1 - Specificity) \times (1 - Prevalence)}$
Negative predictive value	$\frac{Specificity \times (1 - Prevalence)}{Specificity \times (1 - Prevalence) + (1 - Sensitivity) \times Prevalence}$

Formulas for calculating positive and negative predictive values [PPV ,NPV]

The appropriate formulas are shown as . The resulting predictive values are presented in Figure 55. Particularly from the perspective of the country allowing the importation of this dog, in this situation the predictive values of a negative test result are important.

The results suggest that the Dirocheck kit assuming that the sensitivity / specificity values from the second trial are correct, performs rather poorly with a chance of 78% that a negative test result truly is negative.

The Filarocheck kit would appear to provide the best performance, and the sensitivity/specificity data is based on reasonable sample sizes of dog populations.

The possibility remains though that the dog population used in the evaluation of this particular test while large in size may in fact not be representative of the typical characteristics of the dog population in Brisbane

Methods for choosing Normal/Abnormal Criteria

The criteria for deriving cut-off values from diagnostic devices measuring quantities on a continuous scale such as optical densities of ELISA tests can be based on a range of different methods. The most popular technique is called the Gaussian distribution method, but in addition there are the percentile, therapeutic, risk factor, diagnostic or predictive value and the culturally desirable methods.

The Gaussian distribution method is used to derive a cut-off value on the basis of test results from a disease-free population.
The advantage of the technique is that it is simple. But there are many disadvantages. Firstly, the distribution of values is likely to be skewed or bimodal. In addition, it is assumed that prevalence is fixed whereas in reality it will often vary between populations. There is also no biological basis for defining disease on the basis of such a cutoff.

Percentile method, test values are obtained for a large number of disease-free animals, and the lower 95% are classified as normal, the upper 5% as abnormal.

The percentile method is as simple as the Gaussian, but has the additional advantage that it is also applicable to nonnormal distributions. Its disadvantages are otherwise the same as for the Gaussian method.

Therapeutic method, the cutoff value is decided on the basis of the level, at which treatment is recommended. New results from research will allow adjustment of the value. Its advantage is that only animals which are to be treated will be classified as diseased.

A major disadvantage is its dependence on knowledge about therapeutic methods which has to be up-to-date

The risk factor method uses the presence of known causally or statistically related risk factors to determine disease status.

It has an advantage if risk factors are easy to measure and it facilitates prevention. But as a disadvantage, risk of disease may increase steadily (dose-response) which means that it becomes difficult to determine a cut-off. Therefore, the positive predictive value may be low.

The diagnostic or predictive value method which is considered the most clinically sound approach. With this technique, the cut-off is selected so that it produces a desired sensitivity and specificity. This can be done on the basis of the information contained in a receiver operating characteristic (ROC) curve.

The advantages of the predictive value method include that it can be applied to any value distribution.

The disadvantage of the method is that it requires monitoring of prevalence, positive and negative predictive values.

The purpose of a diagnostic test is to influence the clinician's opinion about whether the animal has the disease or not. This means that it is used to modify the opinion (this better be a probability!) which the clinician had before obtaining the test result, i.e. it is a modifier of the pre-test probability. For this purpose, presenting the test result as positive or negative will not be that useful, because the result could still be a false-positive or negative. A quantity is therefore needed which gives the clinician an idea how likely it was that the test result could have been produced by a diseased compared with a non-diseased animal. This can be done with the likelihood ratio.

They can be calculated for negative as well as positive test results. The likelihood ratio for a positive test is estimated on the basis of dividing the probability of a particular test result in the presence of disease (=sensitivity) by the probability of the test result in the absence of disease (=1-specificity). The result is interpreted as how likely it is to find a positive test result in diseased compared with non diseased individuals. The likelihood ratio of a negative test result is calculated as the
quotient between (1-sensitivity) and specificity (see Figure).). It is used less frequently than the one for a positive test. This result is then interpreted as how likely it is to find a negative test result in diseased compared with non-diseased animals.

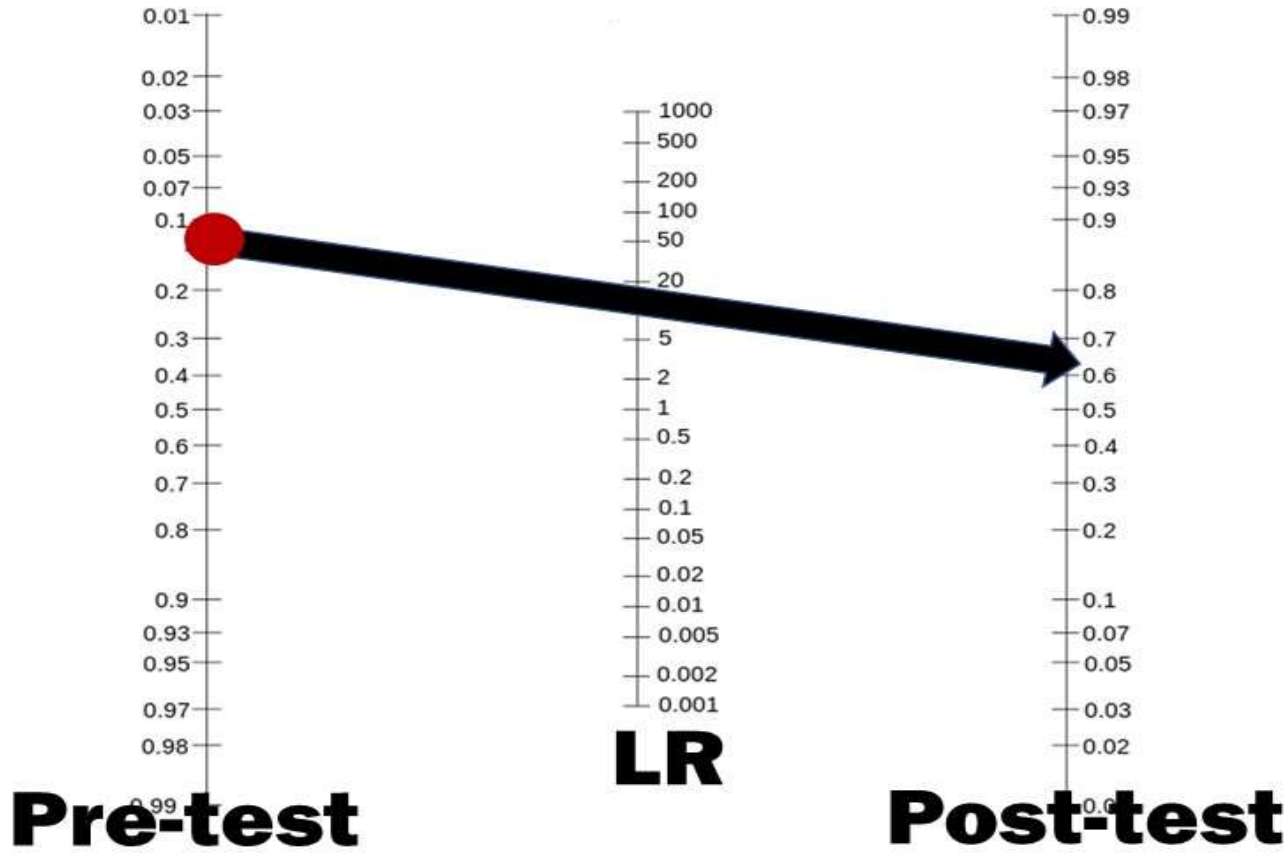
$$LR(+) = \frac{Sensitivity}{1 - Specificity}$$

$$LR(-) = \frac{1 - Sensitivity}{Specificity}$$

Formulas for likelihood ratio LR calculations

Likelihood ratios (LR) do not depend on prevalence, and they provide a quantitative measure of the diagnostic information contained in a particular test result.

Likelihood Ratio



A likelihood ratio is a number used to assess how much impact a test result has on the patient's likelihood of having the disease.

If the test is positive, use the...

$$\text{Positive likelihood ratio} = \frac{\text{sensitivity}}{\text{1-specificity}}$$

If the test is negative, use the...

$$\text{Negative likelihood ratio} = \frac{\text{1-sensitivity}}{\text{specificity}}$$

To calculate the post-test odds of the patient having the disease...

$$\text{Post-test odds} = \text{LR} \times \text{Pre-test odds}$$

Combining Tests

Different diagnostic methods are frequently used in combination n to allow improved diagnosis through strategic decisions about interpretation of results. These approaches include use of different tests for the same disease problem on a single animal, of different tests each identifying different disease problems in a single animal or of the same test for a specific disease problem applied to several animals or the same animal over time.

1-Different tests for the same disease problem in a single animal

2-Using the same test multiple times

3-Using different tests for different disease problems in the same animal