Assay Validation Methods

This document contains educational materials developed by Dr. Larry Hammell, and is used with permission. This information was adapted from sections of the 'Applied Aquaculture Epidemiology' course, developed by the Atlantic Veterinary College and Canadian Aquaculture Institute. The USFWS acknowledges and thanks Dr. Hammell for the use of this material and his contributions to quality assurance in applied aquaculture.

Sensitivity and Specificity

SENSITIVITY is the proportion of true-positives which actually test positive, and how well a test is able to detect positive individuals in a population. A sensitive test will rarely "miss" positive individuals, and should be used when the chance of missing disease poses a large penalty (i.e., introduces a serious or exotic disease).

SPECIFICITY is the proportion of true-negatives which actually test negative, and reflects how well an assay performs in a group of disease negative individuals. A specific test will not produce false positives, or misclassify the identity of a pathogen. A highly specific test should be employed when false-positive results would cause significant impacts to the program (i.e., erroneous reporting of a significant disease in humans, or eliminating rare animals from a broodstock program).

$$\textbf{SENSITIVITY} = \frac{a}{a + c} \qquad \textbf{SPECIFICITY} = \frac{d}{b + d}$$

Table 2. Sensitivity and Specificity formula.

		DISEASE Status	
		D+	D –
TEST Results	Test +	a	
	Test –	c	
		a + c	

	DISEASE Status	
	D+	D –
Test +		b
Test –		d
		b + d

It should be noted that the value for specificity is harder to calculate since defining a disease-free individual is more difficult than detecting a disease-positive individual.

The ideal test is of course both highly sensitive and highly specific. However, the majority of diagnostic tests have continuous outcomes (i.e., quantity of protein, antibody titer, etc.), with the assignment of a positive/negative cut-off value decided by the diagnostician, or authoritative body conducting the testing. This setting of the positive/negative threshold makes these tests appear to produce dichotomous outcomes (i.e., positive or negative).

What occurs when sensitivity or specificity is altered?

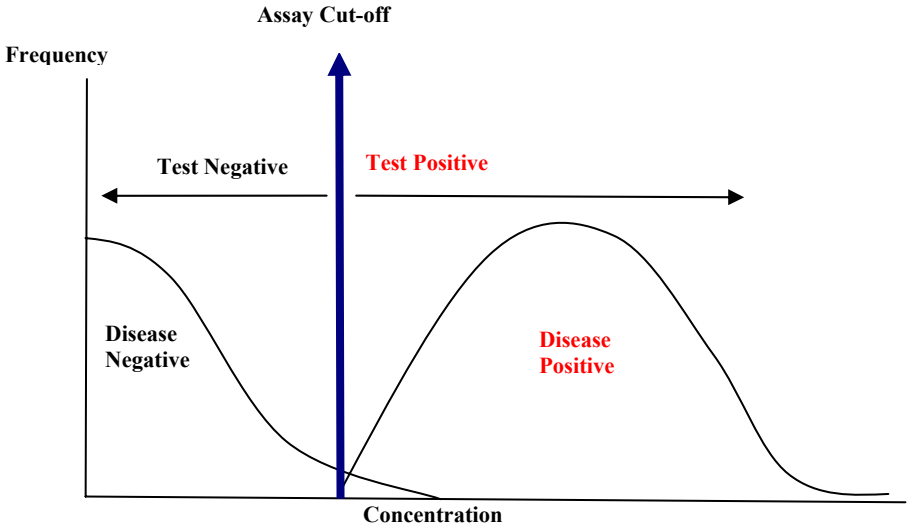
When either test sensitivity or specificity is altered, it will almost always cause a corresponding change in the other. For example, if in a given test, if the cut-off threshold is set lower, to ensure inclusion of all positive fish, it will also include a larger proportion of false-positive test results.

Low Threshold:

All disease-positive fish are included in Positive Test Results:

However, a proportion of disease-negative fish will also be reported in the Positive Test Results;

FALSE POSITIVES



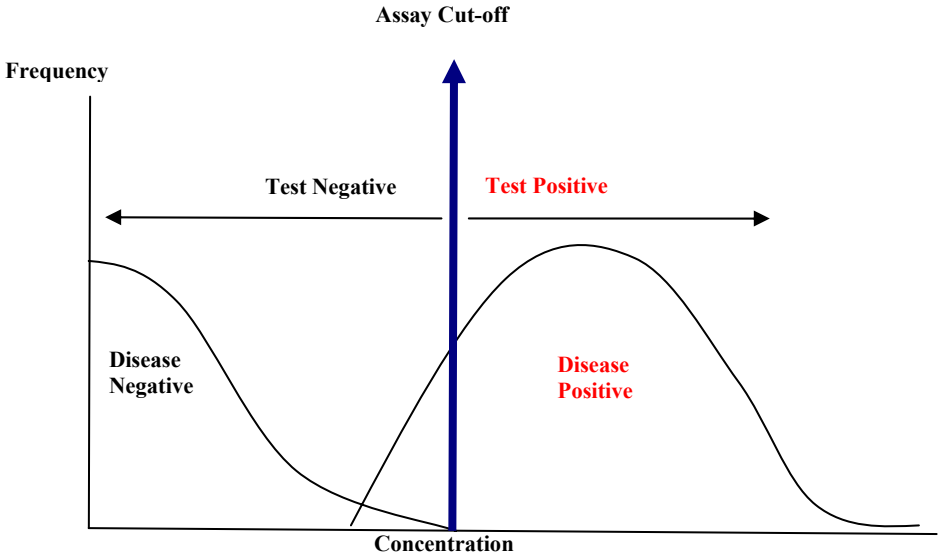
On the other hand, if the threshold is set very high to exclude all negative fish in the positive test results, then the test will also identify a large proportion of true-positive fish, reported as negative test results (false-negatives).

High Threshold:

All disease-negative fish are included in the Negative Test Results:

However, a proportion of disease-positive fish will also be reported in the Negative Test Results;

FALSE NEGATIVES



Determining where to set the cut-off value is very important,
and often is driven by the level of error one is willing to accept in either direction. For example, let's look at the case of a captive breeding program, where every fish is extremely valuable, and as many eggs as possible are needed for the restoration program (i.e., restoration is a priority over disease status of a single pathogen).

In general, as: Sensitivity increases (lower threshold), false-positive test results will increase,
Specificity increases (higher threshold), false-negative test results increase.

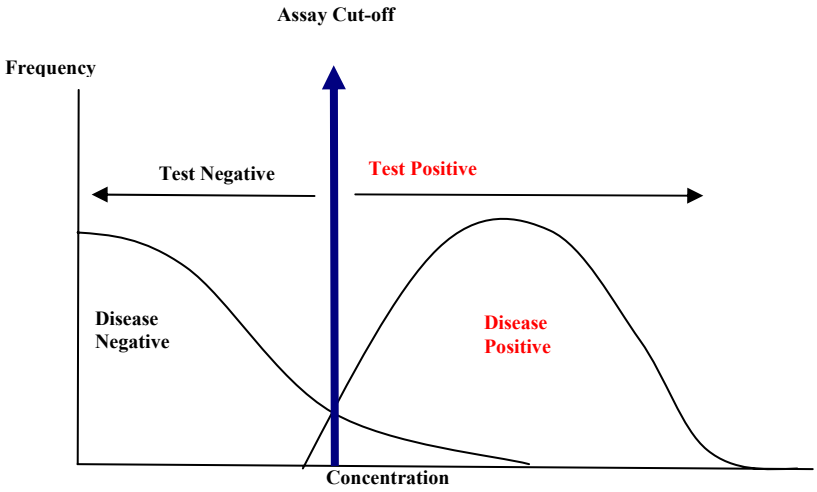
Example: Hatchery practice for salmonid broodstock is to discard all eggs from females testing positive for Bacterial Kidney Disease due to vertical transmission of this disease. In setting up a captive breeding program for recovery of a threatened and endangered (T&E) broodstock, the decision might be made to set the assay threshold

higher for a group of fish already at critically low numbers. By setting the positive/negative threshold higher, you would be ensuring that none of the truly-negative fish are discarded as false-positive fish. The downside of this approach would be the acceptance of some truly-positive fish that will test negative (false negatives). But, as stated previously, this may a risk you are willing to take, versus potentially discarding valuable eggs from negative fish. Genetic diversity (maintenance of as many genotypes in the next generation) usually outweighs disease management in these types of scenarios.

When an assay is validated, through testing the sensitivity and specificity using a known positive population, or spiked positive sample set, the threshold can be set in a balanced manner, or to meet specific objectives. A confidence interval can be constructed (see Evaluating Diagnostic Tests) and the clinician will be aware of the test errors and accepted trade-offs in the testing program.

Balanced Threshold:

Small proportions of false positive and false negative test results cannot be entirely avoided in most disease testing. But the degree of error can be understood through validation testing, and the proportions adjusted to meet the objectives of the testing program.



In review, we have covered the following definitions and looked at examples of how sensitivity and specificity are altered when thresholds are established for a diagnostic test.

Table 4. Review of Testing Terms and Formulas.

		DISEASE Status			
		D +	D –		
TEST Results	T +	a	b	a + b	Apparent Prevalence (a+b) / n
					True Prevalence (a+c) / n
	T –	c	d	c + d	Predictive Value of Positive test a /(a + b)
					Predictive Value of Negative test d /(c + d)
		a + c	b + d	n	Sensitivity a /(a + c)
					Specificity d /(b + d)