Cornell Johne's ELISA (KELA) Performance Characteristics

Category Relative risk of infection	Description of category	KELA value	Associated Sensitivity (%)	Associated Specificity (%)
Low (1-39)	Background activity	0-10	80-90	1.0
		20	70-80	12
		39	60-70	65
Mod (40-54)	2 x Mean *	54	~55	85
Moderate High (55-164)	3 – 10 X Mean	60	50-60	89
		80	40-50	98
		90	30-40	99
		100	30-40	99.5
		120	30-40	99.7
		140	20-30	100
		160	10-20	100
		165	~10	
High (>164)	> 10 x Mean	>164		

98-99% specificity cut-off range (rows 80 and 90)

Table: Sensitivity and Specificity ranges for Cornell KELA ELISA units

* Mean KELA value in animals from NY herds known to be free of Johne's disease infection

** 98-99% specificity cut-off range – 98 to 99% of non-infected cattle will have KELA values below this range (2% are expected to have KELA greater than 80 and 1% greater than 100). That is a 2% and 1% false positive rate respectively.

For further interpretation of the multiple cutoff risk categories see Comments on Performance and Interpreting the Cornell Kinetics ELISA (KELA)

Cornell herd testing strategy

Our general strategy for herd or group testing is to follow KELA testing with fecal culture on cattle with KELA values >40. The proportion of animals in a herd or group in this low risk category ranges from <3% to 50%. The highest observed has been 60%, which was unusual. In known infected herds, typically 15-25% of animals tested will have KELA values greater than 40. Herds with no or low prevalence of infection (Herd Status Program herds) will typically have 3-10% of animals with KELA values > 40, and 2-3% with values > 80.

Validation of the Cornell KELA ELISA

Dr. RH Jacobson developed the Cornell kinetics ELISA test in the early 1990s. A homemade Cornell semi-purified antigen is used. Conjugate is provided by ADRI, Canada and is an anti-IgG1, which is more specific than standard IgG conjugates. For this and other reasons, the Cornell KELA ELISA is not absorbed. Absorption is a difficult procedure to standardize, and results of validation in New York cattle herds indicated it did not significantly change the interpretation of the test. In addition, Cornell always recommends ELISA testing be followed by fecal culture of individuals with elevated values.

This strategy reduces the number of cultures, and thus the cost of testing, by identifying cattle at higher risk of infection based on the KELA for further evaluation. Thus the decision was made to use a non-absorbed ELISA.

The Cornell Johne's KELA has had two phases of validation over the ten-year period it has been used in the New York State Johne's testing program. It has been validated in predominately dairy, and a few beef herds in NY.

Sensitivity of the Cornell KELA – based on two phase validation including culture positive cattle ranging from subclinical to clinical, from 100's of low to high prevalence NY herds:
1990s N= 439 fecal positive cattle
1992-97 N=2342 fecal positive cattle

Specificity of Cornell KELA – based on animals from beef and dairy herds in NY that had no history of Johne's disease, tested multiple times negative by whole herd fecal culture, and achieved several levels of negative status. N=766 cattle.

Diagnostic sensitivity (Se) (relative) definition

In a (finite) population of known ***infected*** cattle used for the validation of a test, sensitivity is the percent of the infected animals that have a value greater than a value of interest (i.e. referring to the whole range of consecutive test values).

If a single cutoff interpretation is used to simplify things, then the Se is the percent of ***infected*** cattle in this population that test (correctly) "positive" – those with a value greater than the "cutoff value". Those below the "cutoff" are considered "negative".

"Relative" sensitivity - for a particular Johne's test or kit, the particular sensitivity value reported for it is really a "relative" one. Simply, this is because there is no perfectly accurate "gold standard" (100% Se and Sp) to compare tests to, and the populations (the composition of infection) used to evaluate tests are essentially "hand picked" by availability and quite different. For example, serology is often validated using fecal culture as the "gold standard" which itself has relatively low sensitivity (~40%)

Reported Se values by researchers and companies will vary depending upon:

1. The "mix" of stages of infection in the validation population.
2. The accuracy of the test used for the "gold standard" to which the test was compared. In this case, which is often the case with validating tests for Johne's disease, the known infected animals were determined because they were positive on fecal culture. The Se estimates then are overestimates of the Se of the tests because animals that are infected and not culture positive (early stages of infection) aren't often included in the validation population. The "overall estimate" of the Se is actually lower.

Thus, the NJWG recommendation to use an estimate of 25%, represents their best estimate of the "average" Se, at least when interpreting the test in lower prevalence situations such as herds testing for the Herd Status Program.

Important point about sensitivity of Johne's tests

As long as the limitations and usefulness of tests for Johne's disease are appreciated, the variation in estimates of sensitivity don't actually have an enormous impact on the final use or interpretation of the test. In other words, whether you use 25% or 50% as a Se estimate does not have a very large impact on a final interpretation of the test results. The prevalence and context of the situation will have a much greater influence on interpretation, and on the decisions based on those test results.

Specificity (Sp) – definition

In a (finite) population of known ***non-infected*** cattle used for the validation of a test, specificity is the percent of non-infected animals that have a value lower than a value of interest (i.e. referring to the whole range of consecutive test values).

If a single cutoff interpretation is used to simplify things, then Sp is the percent of ***non-infected*** cattle that test (correctly) "negative" – those with a value below the "cutoff" value.

Lack of an accurate "gold standard" test also makes it difficult to determine the specificity of Johne's tests. It requires finding truly non-infected cattle, which can only be done by determining that whole herds are not infected, which is difficult.

Specificity indicates how much noise there is in the test in non-infected animals – what to expect for a false positive rate. Specificity, particularly in the case of serology, is influenced by background exposures in the cattle. We know there are other *Mycobacteria spp.* and similar bacteria that may cross-react with Johne's serology. But this has not been studied to any great extent. As more Johne's testing is done, some areas of the country or types of environments can be expected to exhibit more cross-reacting agents and activity than others.

Knowing what to expect for test specificity is most important when interpreting positive responses in animals in relatively low prevalence herds, is it real or not? Using Johne's serology, some false positives should always be expected i.e. at least 2% using a single cutoff interpretation with specificity of 98%.

Fecal culture – organism detection

One great advantage of organism detection tests is that properly performed their specificity is 100%. Fecal culture is the most sensitive organism detection test currently available. Sensitivity overall is estimated at 30-40%. However, rigorous validation of fecal culture suffers from the same lack of a perfect "gold standard" test for comparison and this is the experts' best estimate based on a combination of science and years of use.

One should know the following about the particular Johne's test one is using:

1. It is reasonably "validated" - what population(s) were used, what gold standard was used?

2. Have a "working" estimate of Se and Sp, which may also vary depending on what you know about the stage of infection in the animals being tested. For example, you should consider the Se of Johne's serology to be pitifully low in heifers. In general, they are too young to have antibody. Se will be very high in animals with clinical signs because you are already good at picking them out and if infected most will have antibody and be positive. As for individuals in a herd…..? See 3 below.

3. Have your own estimate of the prevalence of infection in the herd, i.e. the probability that an individual is infected. Because Johne's tests aren't perfect, an existing estimate of probability of infection in the herd, or the individual, is very useful to better interpret a result in an individual. It is more important than the variation in the estimate of Se of the test. Your existing probability estimate of infected or not has a great influence on the additional probability of infected or not that you have after you have added the info provided by the test result. (It is called the predictive value of the test based on its Se, Sp and prevalence).

4. That the laboratory can perform the test with accuracy and repeatability. It should be NVSL approved by the annual proficiency check test.

For more information on interpretation of the Cornell KELA multiple cutoff risk categories see Comments on Performance and Interpreting the Cornell Kinetics ELISA (KELA).

For more discussion on Johne's disease tests, interpreting Johne's test results, and predictive values see NYSCHAP Articles on Johne's Disease, articles 4 through 7.