

Johne's disease in Cattle - Article 5

This article was adapted with permission from the original 1999-2000 series presented by the AABP Food Safety Committee. Content was edited and reviewed by the National Johne's Working Group and endorsed by the USAHA.

Johne's Disease Diagnostic Tests - the ELISA

Prepared and edited by Christine Rossiter and Don Hansen
from the National Johne's Working Group

Current tests are good tools

Diagnostic tests are a tool that helps producers make decisions and reach their goals for prevention or control of Johne's disease. No single test is sufficient for all purposes but reasonably accurate and cost-effective tests are available for different diagnosis and control needs. Beyond confirmation of clinical suspects, carefully consider which tests to use. To choose among available tests, a clear understanding of the following components is important:

- Goals or constraints of the client
- Goals of testing
- Stage of infection
- Accuracy of the test for herds or individuals
- Predictive values of the test
- Cost or impact of false results

Detection of bacteria verses antibody, fecal culture and ELISA

The two most frequently used tests to detect Johne's are the fecal culture and the absorbed ELISA. Fecal culture directly detects intracellular microbes of Johne's disease and indicates infection. Direct detection tests, including fecal culture, are considered the most definitive. Antibody assays, including ELISA, are less definitive in the individual but can provide useful information to assess herd and individual status. These two tests measure infection differently but the magnitude of antibody or organisms cultured tends to correlate with advancing stages of infection.

The Absorbed ELISA

An important step in absorbed ELISA is the addition, before testing, of an extract of *Mycobacterium phlei* to the serum. This reduces cross-reacting antibodies that may produce false-positive results.

ELISA is an absorbed test method. In this partially automated system, antibody in the serum sample binds to *M paratuberculosis* antigen that is attached to walls of the plastic plates. In subsequent steps, with the addition of final substrate, sample antibody that is bound by conjugated anti-antibody colorizes. The amount of color produced is measured at a precise time and wavelength. This numeric value, Optical Density (OD), reflects the amount of antibody in the sample. The OD value defines the sample as positive or negative, relative to a pre-determined cutoff value. See Article 4 for details.

ELISA results

Recall that the accuracy of a diagnostic test is determined by performing the test for sensitivity (Se) on known infected animals and the test for specificity (Sp) on known uninfected animals. The OD values produced by the ELISA range from zero to +10.00, requiring the determination of a cutoff value to separate positive from negative results, i.e., a cutoff value.

Since the laboratories that most practitioners use perform the IDEXX or similar validated tests, reviewing how results are provided is worthwhile. OD value for each sample is first “adjusted” and then compared to the cutoff value. At least one negative (NC) and positive (PC) control samples are run for each plate. At the end of a valid run, the negative control OD value is subtracted from each sample OD value, and from the positive control OD value. The result is then reported as a sample to positive ratio (S/P). ($S/P = S - NC / PC - NC$). The sample S/P ratio is then compared to the pre-determined IDEXX cutoff of 0.25. If the S/P is greater than 0.25, the manufacturer recommends interpreting the sample as positive.

Thinking beyond the single cutoff

More information is available from the ELISA than the positive-negative interpretation. Practitioners should obtain the S/P, PC and NC values from the laboratory to evaluate how sample S/P ratios are distributed around the cutoff. With Johne’s disease, infected animal ratios are spread out on both sides of the cutoff. Non-infected animal ratios tend to cluster around the NC value, but some overlap with infected animal ratios. Animals with ratios below the cutoff, but well above the NC, have a higher likelihood of infection than those with values similar to the NC. Some of these animals are good candidates for closer evaluation and additional testing, including fecal culture. This is especially true when making management decisions where the cost of interpreting the animal’s status as falsely positive is relatively inexpensive, i.e., rejecting colostrum.

Changing the cutoff value, i.e. accepting a lower ratio as a positive result, changes the sensitivity and specificity values. Since Se and Sp are inversely related, lowering the cutoff increases the Se, decreases the Sp, and includes more true positives and false positives. Raising the cutoff lowers the Se, increases the Sp, and reduces the chance of false positive results, but misses some truly positive animals.

Michael Collins, University of Wisconsin, evaluated the current IDEXX Johne’s ELISA in reference serum samples and determined the Se and Sp for several S/P ratios. The Se and Sp are used to determine a Likelihood Ratio (LR) for each S/P. The LR is the odds that a given result is expected in an animal with the disease. The LR for a positive test result is $Se/(1-Sp)$. Using the LR values, he developed a multiple cutoff interpretation using five categories of increasing S/P ratios. This multiple cutoff interpretation utilizes information from the ELISA to reflect the higher number of S/P ratios that occur in cattle with higher numbers of bacteria shed in feces (late Stage II through Stage IV).

ELISA Interpretation Chart for S/P Ratios in Cattle (Spring 2000)

S/P Ratio	Disease status	Comment
0.00 -.09	Negative	No antibody detected. Test again to confirm status.
0.10 -.24	Suspect	May be early stage. Infected ratio is 15 times more likely than negative ratio. Culture or retest in 6 months.
0.25 - .39	Low positive	May be early stage and fecal samples may be positive or negative. Infected ratio is 30 times more likely than negative ratio. Culture or retest in 6 months.
0.40 -.99	Positive	Likely to be in a later stage and shedding. Colostrum, milk and the fetus are at risk. Remove or separate the animals from the herd. Culture samples if confirmation is needed.
1.0 – 10.0	Strong Positive	Likely the advanced stage, with generalized infection and imminent clinical disease. Remove these animals immediately. Culture samples if confirmation is needed.

Reproduced by permission of the author. To be used only in the interpretation of the absorbed ELISA for Johne's disease sold by IDEXX, Inc.

Accuracy of the absorbed ELISA

Accuracy of the ELISA reported by IDEXX is 60% Se and 97% Sp. However, evaluations in other reference populations illustrate that sensitivity varies with the distribution of infection stages in the tested population. Sweeney demonstrated increasing sensitivity with increasing stages of infection. ELISA sensitivity is lowest in subclinical, light shedding cattle (15% ± 6.6%) and highest in clinical cases (87% ± 8.4%). Overall, at the IDEXX cutoff, sensitivity was 45%.

ELISA specificity is reported between 97% and 99%. Practitioners should be aware that cross-reactions may occur with this test in some situations.

Occasionally, individuals with clinical Johne's disease are fecal culture positive, AGID positive and ELISA negative. Why antibody is not detected is unknown.

The ELISA is used in herds that have a mix of animals in various stages of infection. To standardize interpretation of the Johne's absorbed ELISA for the National Johne's Herd Status Program, an expert subcommittee of the National Johne's Working Group created ELISA guideline estimates for Se and Sp values. They recommend a sensitivity of 25%, to account for early infections not represented in many reference populations, and a specificity of 98%.

The influence of prevalence on interpretation of results

The chance that a test result correctly reflects an animal's true infection status depends upon prevalence of Johne's in the herd and the veterinarian's presumptive diagnosis. This reality emphasizes that tests are tools that should be used in the context of other relevant information on infection risk. Please review predictive value and the corresponding table values in Article 4.

The following scenarios are most important to recognize when interpreting ELISA results for mature cattle:

1. In a *low prevalence* environment, i.e., 1%, the chance that a positive ELISA test is correct is 15% to 16%. The chance that a negative ELISA result is correct is 99%.
2. In a *high prevalence* situation, i.e., 30%, the chance that a positive ELISA result is correct is 88% to 90%. The chance that a negative ELISA result is correct is 75% to 85%.

Note: See Article Four, Table 1 for predictive values at different prevalences. Predictive values differ little when sensitivity is between 25% and 60%.

Use of the ELISA

Since the sensitivity of the ELISA is not 100%, a single negative test-result should be interpreted as infection “not detected” versus “completely absent”.

ELISA is not recommended as a stand-alone test for individuals. It is best used to assess herd status. As more information is gained about herd status, the test becomes more useful to measure individuals. Repeat testing on an individual gains additional information.

Most Johne’s disease experts agree that fecal culture should be used to confirm shedding of ELISA positive animals in the following circumstances with:

1. Lack of clinical evidence to support the diagnosis.
2. Uncertain pretest (source herd) prevalence.
3. High cost of a false positive result.
4. Valuable animals.
5. Implementation of a Johne’s Herd Status Program.

Summary of ELISA use for diagnosis

1. For mature-herd testing, the manufacturer reports a sensitivity value of 60% and the NJWG recommends 25%. In practical use, the difference between predictive values or likelihood ratios calculated for these two sensitivity values is minor.
2. ELISA is most useful to measure infection level in mature herds.
3. ELISA is used to assess individual mature cattle suspected of being in late stages of disease and/or of mature individuals in herds with known prevalence.
4. ELISA is not very effective in assessing immature cattle status, i.e., less than 2 years old.
5. The multiple cutoff interpretation offers broader and client-specific use of the information.
6. ELISA is not used for Johne’s-vaccinated cattle since most will test positive.