Johne's testing: What's available?

Here's a look at the main Johne's tests being used and what we can expect tests to tell us.

by Mike Collins, D.V.M.

What tests are now commercially available?

There are two types of tests in routine use. One type measures antibodies in serum (the fluid, noncellular portion of lood). Then there are those that find the organism causing ohne's disease, *Mycobacterium aratuberculosis*, in manure (fecal ulture or polymerose chain reacion [PCR]). In the U.S., there are our commercial, USDA-licensed ntibody test kits: TipTest ImmuCell Corp.); AGID (ImmuCell Corp.); and two ELISAs, one by DEXX Laboratories, Inc., and one rom Biocor Animal Health.

Mike Collins

To test manure samples most labs use traditional culture methods. Labs choose to either make their own culture medium or buy t from a commercial source. There are two commercial ources of the medium for *M. paratuberculosis* culture in the U.S. at present. In addition, a few labs use a commercial, utomated culture system called BACTEC. This system, dapted from technology used to diagnose tuberculosis in humans, involves both a commercial culture medium and a machine that "reads" the cultures.

As an alternative to culturing samples for *M. paratuberculosis*, labs have the option of using a test to find the bacterium's DNA from a manure sample. Such tests are commonly alled "gene probe tests" or PCR tests. PCR tests can be done using either "homemade" kits or a commercial kit.

Confused yet? It gets better (or worse, depending on your iew).

If you include test kits made outside the U.S., the range of roducts is even larger. There are several companies in other ountries that sell diagnostic test kits.

And, there is a third category . . . tests for cellular immuniy. Cellular immunity can be measured by skin testing, just as s done for TB in cattle and humans. Alternatively, you can use a commercial kit together with a blood sample to get esults that basically measure the same thing as the skin est. This test, called the gamma interferon assay, shows coniderable promise but still is under evaluation by research abs around the world.

The **good news** is that this commercial interest in tests for ohne's means that better and better tests are becoming vailable to producers and veterinarians. The surge of new roducts is being driven by investors who see a chance to rofit from selling high-quality diagnostic kits.

The **bad news** is that selection and interpretation of tests or Johne's is becoming more complex. In addition, it takes onsiderable time and money to fairly and objectively evalute these tests head to head. The only fair criterion against which to compare the tests is to assess disease pathology. This means that the animals have to be slaughtered and carefully xamined to decide if the test correctly characterized the animal as infected. You may find it surprising, but few agencies re willing to invest the kind of money needed to do thorough est kit evaluations.

Evaluating tests for Johne's disease is no small feat. It takes a long time and is quite expensive because of the chronic nature of the infection. It makes sense to pool resources and to compare as many tests as possible on the same cows.

Hoard's Dairyman contributed financially to a large effort at the University of Wisconsin, School of Veterinary Medicine to compare multiple Johne's disease tests in the early 1990s. Manure and blood samples were collected from more than 170 cows with confirmed Johne's disease (although they were not yet showing clinical signs of the disease) and 196 cows in Wisconsin certified-free dairy herds.

The serum and manure samples from those cows were saved so that tests developed in the future could also be evaluated. These samples have been shared with commercial and academic researchers around the world. Although the supply of material is almost exhausted, these same samples have been used to evaluate some of the newer tests that came on the U.S. market in the last few years.

How do tests compare in sensitivity, specificity, and turnaround time?

Test **specificity** is a measure of the percentage of time a test result is negative for NONinfected animals (how well the test correctly identifies uninfected animals). Available blood tests for Johne's disease have a high specificity: 97 percent to 99 percent, and culture-based tests are considered 100 percent specific (in other words, no false-positive tests). This generally means that 97 to 99 percent of the time when a blood test is positive, the diagnosis of Johne's disease is right. A positive fecal culture correctly diagnoses Johne's disease 100 percent of the time. (More on profitability later.)

Test **sensitivity** on the other hand is a measure of the percentage of time a test result is positive for infected cattle (how well the test correctly identifies infected animals). Subtracting test sensitivity from 100 percent gives you the percentage of infected cattle missed by the test (false negative). Improving test sensitivity is the biggest challenge for Johne's disease tests due to the biology of this infection. The table below shows test sensitivity on a set of 142 repository serum samples from cows with subclinical Johne's.

	Culture methods		Blood test methods		
	Traditional	Automated (BACTEC)	AGID (ImmuCell)	ELISA (Biocor)	ELISA (IDEXX)
Sensitivity	41.5%	51.4%	26.8%	42.3%	45.8%

Both ELISA sensitivities essentially were the same, twice as high as the AGID. The automated culture method was more sensitive than the traditional culture method. (It was more likely to give a positive result for a truly infected cow.)

The problem with our tests . . .

The pie chart shows the pattern of results for the two most sensitive tests, the BACTEC culture method and IDEXX ELISA.

If you used both tests at the same time on all cows and consider cows to be infected if they test positive on EITHER test, you would correctly diagnose Johne's disease in 61.3 percent of the cows. This figure of 61.3 percent represents the maxi-

mum sensitivity you can get using the two most sensitive ests available at the same time.

The infected cows missed by both tests (false-negative esults) simply were at an early stage of infection when they re not yet producing what the test is looking for. I call these currently undiagnosable" (at the time of testing with commercially available tests). These cows would be detected later such as the following year), however, when they begin to prouce the element looked for by the test (antibody for the blood ests or the organism for culture).

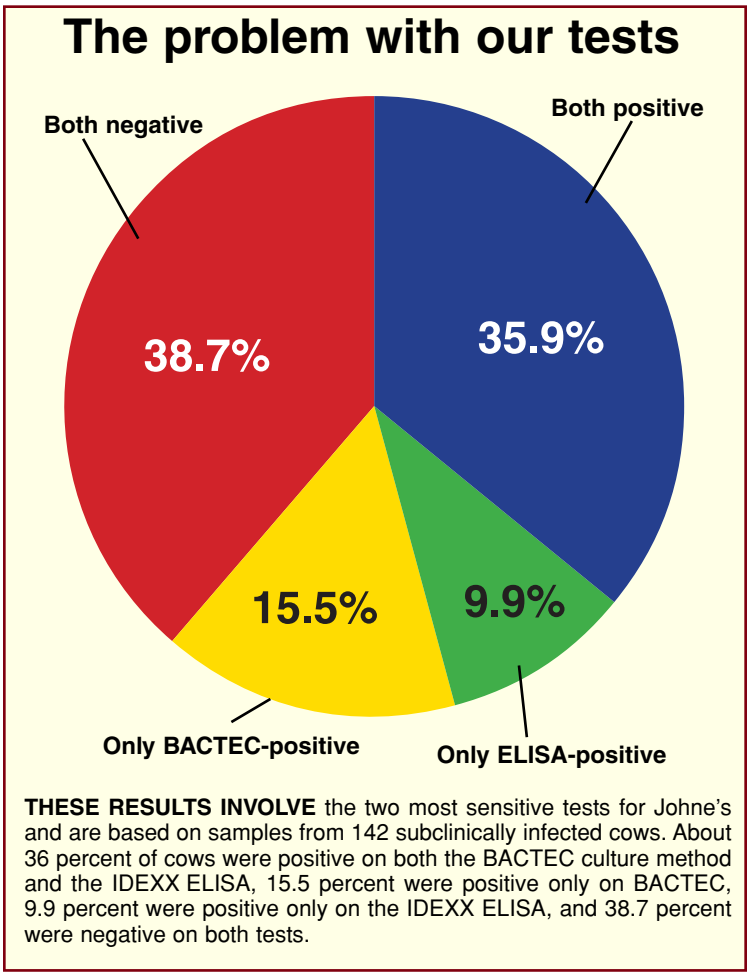
If you consider only the 87 cows that were positive on either he BACTEC fecal culture or ELISA, 74.7 percent of the diagnosable" cases of Johne's disease were ELISA-positive nd over half of them (58.6 percent) also were fecal culture-ositive.

What's confirmatory?

Fecal culture often is called a confirmatory test. Many peole misunderstand what this means. If an ELISA-positive ow also tests fecal culture-positive, you are certain the cow has Johne's disease: the diagnosis by ELISA was confirmed y culture.

However, the information in the pie chart also illustrates an mportant lesson. If a fecal culture done on an ELISA-positive ows is negative, it does NOT necessarily mean the cow does NOT have Johne's. It likely means either that the cow was not shedding the organism that day or that the 3-gram samle collected for testing from the pounds of manure produced hat day did not happen to contain it. On these 87 diagnosble cases of Johne's disease, the culture was false-negative or 14 (16.1 percent) of the infected cows.

What is the best test for your situation? That's where we'll tart in the next installment.



THESE RESULTS INVOLVE the two most sensitive tests for Johne's and are based on samples from 142 subclinically infected cows. About 36 percent of cows were positive on both the BACTEC culture method and the IDEXX ELISA, 15.5 percent were positive only on BACTEC, 9.9 percent were positive only on the IDEXX ELISA, and 38.7 percent were negative on both tests.

What Johne's test results mean

Unfortunately, Johne's testing is not clear-cut. Here's a look at what Johne's test numbers tell you.

by Mike Collins

Is the most sensitive test the best test?

Sensitivity (how well a test correctly identifies infected cows) s important, but there are many other factors to consider. Two re the cost of the test and the turnaround time for results.

For example, fecal culture is two to three times as expensive s the ELISA, and results for culture are not available for two o four months. That compares with just a few days for ELISA.

When selecting a test, consider why you are doing the test nd what you plan to do with the test results. For example, to creen a herd before purchase of some of its cows or heifers, he ELISA gives fast results in a short time. From the buyer's erspective, there is little concern about the few false-positive ests that might happen since the buyer would just choose not o take those animals. On the other hand, owners of herds of egistered breeding cattle may only want to use a test of 100 ercent specificity (no false-positives) to ensure that their herd can be characterized as free of Johne's disease.

How can I use sensitivity and specificity to estimate an nimal's infection status?

Stating a diagnosis in probability terms is useful. It helps ranslate the element of uncertainty that occurs with any iagnostic test into understandable terms. A diagnosis is lways a matter of probabilities.

The probability a test result is correct is called its predictive value. Predictive values are one way to translate the sensitivity and specificity of a test into the probability that a diagnosis is correct.

To estimate the predictive value of a positive or negative test result for Johne's, you first estimate what percentage of the herd is infected with *M. paratuberculosis*. This herd infection rate, called infection prevalence, must be determined for the herd in which the animal was raised to best reflect the animal's exposure to the infection.

This is best illustrated with an example. Consider how the ELISA (sensitivity = 45.8 percent and specificity = 98 percent) performs in two different herds, A and B. Herd A has a true *M. paratuberculosis* infection prevalence of 1 percent and herd B has a true infection prevalence of 10 percent.

The table below shows the predictive values (probability the test result is correct) for both positive and negative test results.

As you can see, the predictive value of a positive result is

Predictive values of ELISA results

	Herd A (1% prevalence)	Herd B (10% prevalence)
ELISA-positive	18.8%	71.8%
ELISA-negative	99.4%	94.2%

higher for a herd with a reater known prevalence of ohne's.

In Herd B, the probability a ositive ELISA is correct 71.8 percent) may be high nough to satisfy you of a ohne's disease diagnosis. You an save money by not doing a ecal culture and simply ulling the cow at the end of her lactation.

However, in Herd A, the robability the ELISA is corect is not that high (18.8 perent). A herd owner either an try a fecal culture on the ow to determine if she is hedding *M. paratuberculosis* r simply record the result in he cow's record, keep an eye on her for signs of Johne's, and etest her next year. Negative predictive values are high for oth herds, but the owner of Herd A is more confident that the ELISA-negative cows are not infected than is Herd B owner.

Predictive values were favored among laboratory diagnostiians in the mid-90s. They have been replaced by a new conept in test interpretation called likelihood ratios.

Some labs report a number with my ELISA results. Is it mportant?

ELISAs measure the amount of antibody in blood. Research t the UW School of Veterinary Medicine has shown that the mount of antibody is directly related to the probability of *M. aratuberculosis* infection. Although ELISA results are typially interpreted as "positive" or "negative," the numerical esult is very important information.

For the ELISA used most widely in the U.S., this number is alled the "S/P ratio." It simply is a comparison (ratio) of the esult found on the test sample (your cow's serum) to positive nd negative controls provided in the test kit (S/P refers to Sample/Positive).

The official cut-off between negative and positive results is n S/P ratio of 0.25. Results equal to or higher than this are lassified as positive, and those below 0.25 are considered negtive. A strength of this interpretation system is that all samles run in any lab with this kit (IDEXX) can be compared ince the same positive and negative control samples are used with every kit. It also provides stability in the assay when omparing results between tests run on different days or by ifferent technicians.

What the S/P can tell us . . .

At the University of Wisconsin, we compared ELISA S/P alues for 142 cows with confirmed cases of subclinical ohne's disease and more than 3,000 cows from certified ohne's-free herds. For each ELISA S/P level, we calculated the percentage of true Johne's cases above that S/P level (sensitivity of the ELISA at that S/P) and the number of Johne's-free cattle above that S/P level (false-positive results if that S/P cut-off was used). If we divide these two numbers, we get a ratio (called a likelihood ratio). This ratio is basically the comparison of the frequency of correct to incorrect diagnoses for each level of ELISA S/P. The math is simple to do and not so simple to explain. Showing the results in a table makes it easier.

What an ELISA Johne's report looks like

Test Performed: ELISA

Tube#:	Animal ID:	Result:	S/P Ratio:
1	551	Strong positive	2.1
2	B1084	Suspect	0.12
3	B135	Strong positive	1
4	1176	Negative	0.04
5	1112	Suspect	0.14
6	1132	Suspect	0.17
7	B117	Negative	0
8	B793	Low Positive	0.26
9	B143	Negative	0.04

A likelihood ratio is the odds of finding this ELISA S/P result in a cow **with** Johne's, compared to cows that are **not** infected.

ELISA S/P value	Ratio of correct to incorrect diagnoses at this S/P level	Conversion of likelihood ratios to probabilities
0.15	6:1	84.8%
0.25	16:1	94.3
0.50	65:1	98.5
0.75	148:1	99.3
1.00	265:1	99.6

The most important lesson to learn from this is: knowing the ELISA S/P value is important. Higher values indicate a greater chance the animal tested is truly infected. Ask your veterinarian and diagnostic laboratory for this information.

Astute readers will quickly ask why the probabilities listed in this second table are different, and generally higher, than those in the first table of predictive values for Herd A and Herd B.

First, the second table only lists likelihood ratios and probabilities for being infected when the ELISA is above each S/P value listed. This compares to the predictive value of a positive test (S/P above 0.25) in the first table. Second, these probabilities have not been adjusted for specific herd infection prevalence. These are essentially predictions for any cow (2 years or older) from any herd at any time.

This illustrates a nice feature of likelihood ratios: You can use them to interpret any ELISA on any cow without having to know, or guess at, the prevalence of Johne's disease in the herd where the animal was raised. However, if you do have Johne's disease prevalence information, you can use it to calculate more herd-specific infection probabilities before and after running the ELISA.

What's ahead in Johne's testing?

Someday we may see a milk tank herd test for Johne's or a skin test for 6-month-old heifers.

by Mike Collins, D.V.M.

IN A previous article (April 10 issue, page 255), I used the examples of two herds: Herd A (low infection prevalence of 1 percent) and Herd B (higher infection prevalence of 10 percent). Let's look at these two herds again to show how ELISA S/P (serum/positive) results affect the probability of getting a correct diagnosis.

	Herd A	Herd B
	Herd infection rate*	
	1%	10%
ELISA S/P	Probability a cow is infected at each ELISA S/P value	
.20	9.7%	54.3%
.40	25.6	79.1
.60	40.3	88.1
.80	52.2	91.3
1.00	61.3	94.6

*Herd infection reflects the probability that a cow is infected BEFORE we know her test results.

As shown, the probability that a cow is infected goes up as the ELISA S/P increases. However, it only goes very high if the herd has a significant infection rate. Said another way, false-positive blood tests are more common in herds with no history of Johne's or those with very low or close to zero infection rates.

There are several advantages to this method of using ELISA results:

- There is no black and white classification of results as being positive or negative.
- The herd owner can make his or her own decision on how to manage the cow.
- Both herd infection prevalence and magnitude of the ELISA result are considered in the diagnosis probability.

Is there any relationship between the ELISA S/P and the chance the cow will be fecal culture-positive?

Yes. In fact, the ELISA S/P relates well to all other diagnostic tests. The table below shows test results for the 142 cows with confirmed *M. paratuberculosis* infections grouped according to ELISA S/P result.

		Percentage of cows testing positive by each test			
ELISA (IDEXX) S/P range	Number of cows	Traditional fecal culture	BACTEC fecal culture	AGID (ImmuCell)	ELISA (Biocor)
0.00 - 0.10	64	20.3%	26.6%	0.00%	4.7%
0.11 - 0.24	13	46.2	38.5	15.4	30.8
0.25 - 0.39	9	22.2	33.3	22.2	44.4
0.40 - 0.99	15	33.3	66.7	20.0	66.7
1.00 or above	41	80.5	92.7	75.6	95.1

I have heard that ELISA testing the same sample twice does not always produce the same result.

Any test that gives a numerical result has some measurable error rate. For example, when news agencies run public opinion polls, they say that the estimated percentage of people responding "yes" to a question is, for example, 40 percent ± 4 percent. This means the true value falls somewhere between 36 percent and 44 percent.

ELISA S/P values also have some measurable degree of variability. This means the same sample of serum tested twice may not yield exactly the same result. Users of the test need to know how much variation in the S/P result to expect.

The Johne's Testing Center at the School of Veterinary Medicine just completed analysis of ELISA repeatability on 180 serum samples with ELISA S/P values ranging from 0.25 to 2.26. Here is what we found.

When the same sample was tested twice on the same day in the same lab, the ELISA S/P average variation (called standard deviation by statisticians) between tests was ± .02 S/P units. When the same sample was tested on two different days, the ELISA S/P variation was ± 0.09 S/P units.

Recently, a veterinarian split 15 blood samples into two parts and sent them to two Wisconsin labs. The average difference in ELISA results across all 15 samples was 0.02 S/P units. For the majority of samples, these differences would not change the interpretation of the test.

We also evaluated ELISA repeatability when 32 cows were blood sampled twice, a month apart. The ELISA S/P results on the two tests varied, on average, only ± 0.09 S/P units. Some may think this means the ELISA is not very reproducible. However, I think this is typical for assays of this type and perfectly acceptable as long as clients are told that any ELISA S/P value reported is basically accurate ± 0.09 S/P units.

The National Johne's Working Group is in the process of conducting a similar study involving a standard set of serum samples tested regularly for six months in five labs in the country.

Aren't there any tests that give a straight-ahead answer?

Sure, if you do not like diagnostic probabilities but prefer diagnostic certainties, then fecal culture is the test you want. Barring rare laboratory contamination or some on-farm mistake in sample collection, a positive fecal culture nearly always means the cow is infected.

Why blood test for Johne's then?

Cost and speed. A blood test for Johne's costs a farmer $8 to $12, and the results are available in less than a week. A fecal culture costs $15 to $25 or more and takes three or four months. Many laboratories are so busy that you must schedule submission of fecal samples up to six months in advance.

In my opinion, for most herds, owners get the most "bang for the buck" by using the ELISA, recording the S/P value in cow records, and using this data to make culling decisions. The ELISA is a Johne's management tool. Use it correctly and use it together with good calf rearing practices, and I'm confident you can control or even eradicate this infection from your herd.

We need to get away from positive/negative ELISA interpretations. They just cause arguments about false-positive and false-negatives. Instead, we should use the ELISA S/P as a gauge of the probability of *M. paratuberculosis* infection and allow herd owners to make their own decisions on how to respond to the result.

Which laboratories are the best?

Thanks to the National Johne's Working Group, a program is now in place that gives users confidence that the laboratories they use are competent to run tests for Johne's disease. The program is administered by the National Veterinary Services Laboratory (NVSL), a USDA agency in Ames, Iowa.

Thirty-nine labs are on the approved list for fecal testing in 2001, and 62 passed the ELISA proficiency test. These labs are listed on a website (http://www.aphis.usda. gov/vs/nvsl/). Be sure your veterinarian sends samples from your herd to an approved laboratory.

Will there be new tests in the near future?

My guess is that a test for antibodies or the *M. paratuberculosis* bacteria on bulk tank milk may soon be available. I also think that the skin test of gamma interferon assay may be proven sufficiently accurate for use to detect infected heifers.

While I am very optimistic about the probability of better tests becoming available, I am also confident that we have sufficiently good tests right now to deal effectively with this disease.

So, should I test and by which test?

Yes. If your herd is infected, you need to know it as soon as possible and get started on a control plan. I compare this to cancer screening. The objective is to make the diagnosis early while the problem can still be treated.

If your herd is not infected, you should verify this by testing and turn this into a marketing advantage when selling cattle.

The choice of tests is straightforward in my opinion. You get the most bang for the buck by using the ELISA first and following up with fecal culture when needed.

This program is less than half the cost of doing fecal culture first for most all herds. Using testing costs of $10 per head for ELISA and $25 per head for culture, the table below compares total costs, for herd owners who decide to test just 30 cattle, for two testing strategies: ELISA first versus culture only.

	Testing options for 30 cows	
Testing outcome	**ELISA then culture**	**Culture only**
100% cows test negative	$300	$750
10% cows test ELISA-positive; culture follow-up	$375	$750

Critics will say the testing costs I used are not accurate. It is true that some states heavily subsidize testing. In this case, the comparisons are different. Other critics will say that the ELISA is missing some fecal shedders allowing the problem to spread. My argument is that culture misses some, too, and that Johne's disease control for infected herds is a four- to seven-year program. You cannot afford to identify and cull 100 percent of infected cattle. Take it slow, and make it affordable using the fast and low cost ELISA as a tool.

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