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Johne's Direct Fecal PCR Fact Sheet for Veterinarians

The Animal Health Diagnostic Center (AHDC) at the College of Veterinary Medicine, Cornell University in partnership with the NYS Department of Agriculture and Markets (NYSDAM) is now offering a Johne's Direct Fecal PCR test. This test is available to veterinarians and is intended to replace individual cow cultures for most herds controlling Johne's Disease through management interventions and testing.

What sample is needed?

At least 15 grams of bovine feces from an individual animal. Environmental manure samples, tissue or milk samples, non-bovine fecal samples, or composite samples of multiple cows are not acceptable. Fecal cups sold by our diagnostic supply center, filled half-full, are ideal for handling and shipping. Containers must be leakproof. Keep refrigerated until shipped overnight on ice. Ship these samples as UN3373 Biological Substance Category B.

What is the cost of this test?

The cost to the veterinarian for samples submitted directly to the AHDC is $11 per test for NYSCHAP herds and $15 for other NY herds. The cost is $18 for out-of-state herds. Samples must be submitted through a licensed veterinarian.

How will results be reported?

Results will be reported through the normal channel to the veterinarian. For NYSCHAP herds, the results will also be available on the NYSCHAP database used to access other NYSCHAP-related results. Veterinary clients can set up on-line access to their results at
http://diagcenter.vet.cornell.edu/dlaccess/login.asp

What is the lag time?

Results will normally be available 7-10 business days after the AHDC receives the sample.

Is advanced scheduling required for this test?

Yes. The lab should be called (Johne's Lab, Monica Carey, 607-253-4473) in advance of collecting samples for this test, to schedule testing. Single clinical suspect samples do not require advanced scheduling. The AHDC anticipates being able to meet testing needs for this new assay, but large numbers of samples and a brand new testing platform and procedures will require scheduling for a transition to full adoption of this test by our clients.

Please bear with the lab as we roll out this advanced assay to serve you better. Multiple quality assurance steps have been built into the protocols for this new assay so that we can carefully monitor performance to make sure this assay performs up to the standards necessary to meet your needs.

What are the details of the test?

The Johne's Direct Fecal PCR test, is a real-time polymerase chain reaction assay with an internal amplification standard control for every sample. It is validated for high throughput in a 96-well plate format. It detects amplified DNA of *Mycobacterium avium* subsp. *paratuberculosis* extracted from fecal samples of shedding cows. PCR assays are run in thermal cyclers, which produce results as the numbers of cycles to positive (Ct) versus not detected. Validation of this assay included a quantitative evaluation of

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the relationship between cycles to positive and the number of colony counts (CFU) on solid HEY agar culture, which has been used as the gold standard for MAP detection. Since a statistically significant relationship exists between Ct and CFU, a quantitative result will also be reported, suggesting shedding at the "Light", "Moderate", or "Heavy" level.

In what situations might this test be used?

This test may be used in a herd control program, and it may also be used as a primary diagnostic test in individual cows with clinical signs compatible with Johne's disease. As has always been the case, Johne's disease needs to be approached by first addressing the areas of management that allow susceptible animals to be exposed to an infective dose of MAP. An overall Johne's disease control strategy and herd health plan should be in place for a given herd. For more information on Johne's disease control strategies see: http://www.nyschap.vet.cornell.edu/module/johnes/johnes.asp. Diagnostic testing is only one piece of the control strategy aimed at identifying animals that are shedding or have the potential to shed MAP at a time when they would expose susceptible animals such as at parturition.

High sensitivity, rapid turnaround and reasonable fees, even for non-NY cattle, will make this test the most appropriate test for clinical suspects. Clinical Johne's disease, which occurs in advanced MAP-infected cattle, is accompanied by heavy shedding. This PCR assay has detected 100% of all heavy shedders in two different validation sample sets of 601 and 804 cows from high prevalence Map-infected herds, with 17 and 58 heavy shedders, respectively.

Removal of heavy and moderate shedders has been identified as important in helping to reduce transmission of MAP within herds. The current recommendation of the AHDC and NYSDAM is to consider using this test as an individual cow level test to detect heavy and moderate shedders. Internal validation with 804 samples collected in high prevalence populations estimates the relative sensitivity of this direct fecal PCR assay for detection of heavy and moderate shedders, as compared to solid HEY agar culture, at 95.45%. For light (few) shedders, it is 74.43%*. Therefore, herds managing light shedders by immediate culling may want to continue to use culture methods. Specificity of the assay components have been determined to be 100% on an NVSL proficiency test with known negative samples.

Validation work has not been completed for pooled fecal PCR detection, or for PCR detection in environmental samples, non-fecal specimens, or non-bovine species. Some regulatory/export agencies may not allow substitution of the fecal PCR for accepted culture procedures.

This test will be the default test for individual sample testing of bovine samples comprising positive pools as detected by culture. This will improve both the cost and turn-around time for pooled testing when positive pools are detected.

Can this test be used in the National Voluntary Johne's Test Negative Status Program (TNSP)?

Yes. The AHDC has passed the USDA Johne's PCR checktest. Direct fecal PCR testing is one of the approved methods for testing in TNSP herds. In the USDA program standards, this test is referred to as a "DNA probe."

Can the values for the direct fecal PCR be compared to the liquid culture?

The AHDC conducted a study comparing direct fecal PCR, liquid culture, and solid HEY agar culture on 804 samples in 2009. Data have not yet been summarized for liquid culture results, but a statistical correlation exists between PCR and solid HEY culture. Previously, the AHDC has internally validated liquid culture in comparison to solid HEY culture, demonstrating a similar statistical correlation. It appears that the PCR assay will perform similarly to culture in detecting heavy and moderate shedders.

Since culture also has limitations in sensitivity, relative to detecting all shedding or infected cows, it is difficult to determine which testing platform has the best sensitivity in detecting all shedding cows*.

How do I interpret the test results on an individual cow?

A "not detected" result does not necessarily indicate that the cow is not infected or not shedding as evidenced by the sensitivity of 74.43% on a population of cows positive on fecal culture. False or unconfirmed positive results are also possible when single samples are tested by different assays, as there may be within-sample variation in the distribution of the MAP organisms. The primer set selected by the reagent manufacturer is expected to be specific to MAP, and has undergone testing to assure non-detected results when tested against known negative samples provided in the NVSL proficiency test. The AHDC has not evaluated the performance of the assay in advanced test-negative status herds.

For more information on Johne's disease diagnostic testing strategies please talk with your NYSDAM field veterinarian or contact the AHDC and speak with Dr. Belinda Thompson (607-253-3908) or Dr. Paul Virkler (607-253-3892). Also see: http://ahdc.vet.cornell.edu/docs/Johnes_Disease_Program_Sampling_and_Testing.pdf. For Johne's disease control strategies see: www.nyschap.vet.cornell.edu/module/johnes/johnes.asp.

*It is possible that this PCR assay may actually have superior sensitivity in detecting shedding, as compared to current culture techniques. Long-term study of infected animals, or other techniques, such as evaluation compared to detection in post-mortem tissue via multiple techniques, would be needed to define sensitivity greater than that detected by culture.

Selected references:

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2. Alinovi CA, Ward MP, Lin TL, Moore GE, Wu CC. Real-time PCR, compared to liquid and solid culture media and ELISA, for the detection of Mycobacterium avium ssp. paratuberculosis. *Vet Microbiol.* 2009 Apr 14;136(1-2):177-9.

3. Clark DL Jr, Koziczkowski JJ, Radcliff RP, Carlson RA, Ellingson JL. Detection of Mycobacterium avium subspecies paratuberculosis: comparing fecal culture versus serum enzyme-linked immunosorbent assay and direct fecal polymerase chain reaction. *J Dairy Sci.* 2008 Jul;91(7):2620-7.