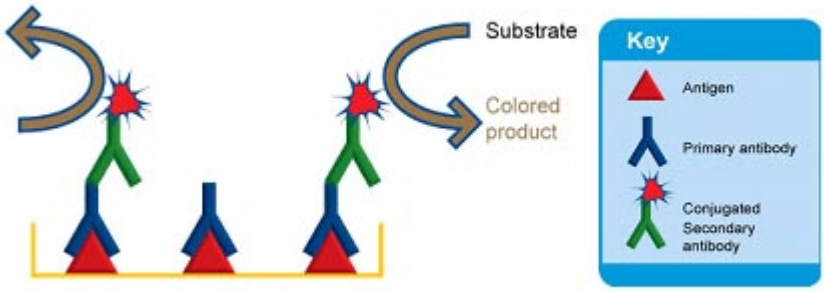
Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Johne's Disease Interpretations

Johne's Disease Antibody ELISAs:

Valuable diagnostic information can be gained from a quantitative interpretation of the Johne's ELISA. In general, the ELISA value is a measure of the concentration of serum antibodies to *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Generally, serum antibody levels increase as the infection progresses. Animals with higher ELISA values are more likely to be shedding the bacterium in milk and colostrum and be heavy fecal shedders than lower scored animals. High ELISA scored animals are also at increased risk of developing clinical Johne's disease.

Currently, there are three, USDA-certified kits available in the U.S. for the diagnostic detection of MAP-specific antibodies. The VMRD *Mycobacterium avium paratuberculosis*, Zoetis Paratuberculosis SERELISA kit and the IDEXX Laboratories, Inc MAP Antibody ELISA. The WVDL is currently providing and is proficiency tested using the VMRD kits for the detection of MAP specific antibodies. WVDL does not currently use the Zoetis Paratuberculosis SERELISA kit or the IDEXX Laboratories, Inc MAP Antibody ELISA kits. It is important to remember that each kit manufacturer develops proprietary reagents such as antigen and conjugates (antibodies) that may not be the same. It is possible that one serum/plasma sample could test positive with one kit, but be negative with the other. This is because the antibody in the serum/plasma may only bind the one antigen or antigenic site from one kit manufacturer, but not the other kit manufacturer's antigen. The only way to confirm if the animal is infected with MAP is to send a fecal sample for direct PCR or liquid culture.



Abcam.com

VMRD MAP Antibody ELISA:

The following numerical scoring system is designed to aid in the clinical management of Johne's disease in tested herds using the VMRD MAP Antibody ELISA assay. Interpretation of individual animal results should be done by the herd veterinarian in conjunction with a thorough consideration of on-farm management practices, herd history for Johne's disease and concurrent testing information gathered from several animals in the herd. The VMRD MAP Antibody ELISA is approved for use with bovine serum and plasma, caprine serum and bovine milk samples.

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For bovine serum and plasma samples:

S/P & Interpretation	Explanation & Recommendation
0.0 - 0.295 Negative	Antibodies to *M. paratuberculosis* were not detected. In *M. paratuberculosis* infected herds, a number of animals may be fecal culture or PCR positive, but ELISA negative. A negative fecal culture or PCR test in 6-12 months will increase confidence the animal is free of infection.
0.296 - 0.395 Suspect	Cattle with ELISA results in this range are more likely to be *M. paratuberculosis* infected than the ELISA negative animals. Retesting these animals in 30-60 days is recommended. Animals that remain inconclusive on retest should have a fecal sample submitted to a diagnostic laboratory for a fecal culture test.
> 0.395 Positive	Antibodies to *M. paratuberculosis* were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with *M. paratuberculosis.*

For bovine milk samples:

S/P & Interpretation	Explanation & Recommendation
0.0 – 0.125 Negative	Antibodies to *M. paratuberculosis* were not detected. In *M. paratuberculosis* infected herds, a number of animals may be fecal culture or PCR positive, but ELISA negative. A negative fecal culture or PCR test in 6-12 months will increase confidence the animal is free of infection.
> 0.125 Positive	Antibodies to *M. paratuberculosis* were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with *M. paratuberculosis.*

For caprine/ovine serum samples:

S/P & Interpretation	Explanation & Recommendation
0.0 – 0.795 Negative	Antibodies to *M. paratuberculosis* were not detected. In *M. paratuberculosis* infected herds, a number of animals may be fecal culture or PCR positive, but ELISA negative. A negative fecal culture or PCR test in 6-12 months will increase confidence the animal is free of infection.
> 0.795 Positive	Antibodies to *M. paratuberculosis* were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with *M. paratuberculosis.*

The VMRD MAP Antibody ELISA test kit is licensed by the USDA APHIS VS.

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Johne's Direct Fecal PCR:

At the WVDL, we use an in-house validated Direct Fecal PCR that detects *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) extracted from fecal samples using three different genes including IS900, the most recognized gene target for MAP. An interpretation protocol has been established based on over 2,500 diagnostic samples of multiple different species in order to utilize these three targets to provide the most comprehensive MAP detection available. In order for a sample to be considered positive, two of the three targets must be detected in the multiplex PCR in which one must be IS900. The IS900 target Ct is the value that is reported on the result report since this target is the most sensitive due to being present in multiple copies in the genome. The validation completed at WVDL has shown that the combined use of these three targets in one assay is a reliable tool for MAP detection and limits cross-reactions from members of the *Mycobacterium avium* complex.

For a small subset of single samples (less than 0.01%), particularly for exotic samples, the interpretation using a three-target multiplex PCR could not accurately be determined. This data, combined with the animal histories, required us to create a new test interpretation of "**undetermined**". The test result of undetermined will be entered on a result report based on our validation criteria and WVDL recommends retesting this animal within six months.

Fecal samples can be pooled in groups of five of the same species. Pools are **made at the laboratory and all positive pools are automatically run** individually in order to identify the positive sample(s). Pools comprised of multiple days collected from the same animal will not be tested individually if the pool is positive unless specified by the submitter.

The test may be used in a herd control program and may also be used as a primary diagnostic test for individual animals with clinical signs suggestive of Johne's disease.

Ct Value	Explanation & Recommendation
Ct value ≤ 23.0	Strong positive reaction indicative of an abundance of MAP nucleic acid
Ct value 23.1 to 32.9	Moderate positive reaction indicative of moderate amounts of MAP nucleic acid
Ct value 33.0 to 37.0	Weak positive reaction indicative of small amounts of MAP nucleic acid
Ct value 37.1 to 40.0	Inconclusive. There is low confidence that Ct values in this range can be distinguished from a negative result. A follow up MAP PCR or fecal culture test is recommended.
No Ct value	Undetermined. A Ct value was obtained from one target or IS900 was not positive. A follow up MAP PCR or fecal culture test is recommended.
No Ct value	No MAP detected

A "not detected"/undetermined result does not rule out the possibility that an animal is infected. A fecal sample from an animal shedding MAP in very low numbers may fall below the limits of detection for the test. Interpretation of individual animal results should be done by the herd veterinarian in conjunction with a thorough consideration of on-farm management practices, herd history for Johne's disease and concurrent testing information gathered from several animals in the herd.

If a positive result is obtained on what is believed to be a negative animal, it is suggested to proceed in the following manner to confirm or deny the positive: 1) ask for Johne's liquid culture (49-day minimum test) if time permits followed by PCR, 2) ask for a retest of the same sample, 3) collect a new sample and resubmit.

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Johne's Liquid Culture:

At the WVDL, we have the capability of using a culture system for the replication and detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP). This test is mostly used for regulatory purposes and the WVDL recommends the PCR to be used when the testing is not for a regulatory purpose. Other reasons the test may be used include: the submitter requires a live organism, the submitter suspects a member of the *Mycobacterium avium* complex that is not MAP, or if the submitter requires that the sample be cultured to confirm viability. The liquid culture will be confirmed by real time PCR. Data supports that the PCR and liquid culture are approximately equal in sensitivity and some literature suggests that the PCR is more sensitive. Liquid culture requires extensive incubation periods and is not ideal for diagnostic testing. Therefore, the WVDL recommends the fecal PCR rather than liquid culture. The liquid culture system uses feces diluted in sterile water, followed by two overnight enrichments in broth with antibiotics to enrich and select for *Mycobacterium*. Therefore, there is a three day setup period prior to incubation. Samples are only setup on Monday, Tuesday and Wednesday. Afterwards, the sample is mixed with proprietary media, as described by the manufacturer, and incubated in capped bottles for 42-49 days for bovine samples and 49-56 days for caprine, ovine and other species samples.

Liquid Culture Reported Results:

No M. Avium paratuberculosis detected, no MAP detected by either liquid culture or PCR
*****M. Avium paratuberculosis detected,** MAP was detected by culture and confirmed by PCR.

References:

1. Collins MT (2002) Clin Diagn lab Immunol 9: 1367-1371.
2. Collins MT, Wells SJ, Petrini KR, Collins JE, Schultz RD, Whitlock RH (2005) Clin Diagn Lab Immunol 12: 685-692.
3. Collins MT, Gardner IA, Garry FB, Roussel AJ, Wells SH (2006) U Am Vet Med Assoc 229: 1912-1919.
4. Collins MT, Eggleston V, Mannin EJ (2010) J Dairy Sci 93: 1638-1643.
5. Villarino MA, Scott HM, Jordan ER (2011) J Anim Sci 89: 267-276.
6. Collins MT, Sockett DC (1993) J Am Vet Med Assoc 203: 1456-1463.