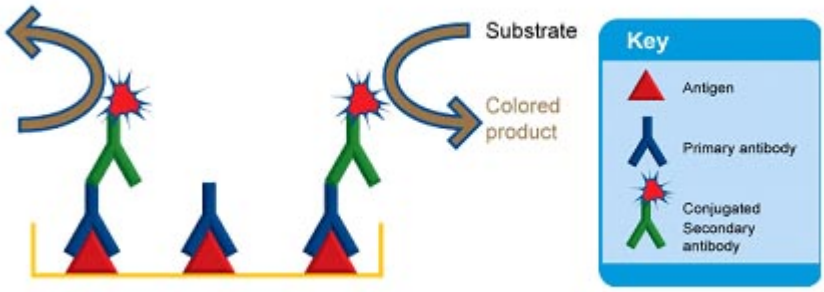
Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Mycobacterium avium paratuberculosis (Johne's Disease) Interpretation Guidelines

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 Disease Direct Fecal PCR:

At the WVDL, we use an in-house validated Direct Fecal PCR that detects the presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in fecal samples using three different gene targets including the IS900, the most recognized gene target for MAP. This assay provides the most comprehensive MAP detection base on results interpreted using the combination of these three targets in one assay. Based on over 2,500 diagnostic samples of multiple different species, our data shown that this multi-targets approach is a reliable tool for MAP detection and limits false positive from other members of the *Mycobacterium avium* complex.

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 the IS900 target, since this target is the most sensitive with its presence in multiple copies in the genome. The Ct value for the IS900 target is the value that is reported on the report. The result is deem 'Undetermined' when only IS900 target was positive or 2 targets other than IS900 were positive, this has only occurred to a small subset of samples (less than 0.01% of samples tested at WVDL), particularly from exotic animal species. WVDL recommends retesting this animal with a follow up PCR or a Johne's Disease Liquid Culture.

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.

***Mycobacterium avium paratuberculosis* PCR Interpretation**

Ct Value	Interpretation	Explanation
Ct value ≤ 36.9	Positive	Positive reaction indicative of the presence of target nucleic acid.
Ct value 37.0-40.0	Inconclusive	Weak positive reaction indicative of small amounts of target nucleic acid which could represent early or late infection, residual vaccine or environmental contamination. -A follow up MAP PCR or fecal culture is recommended.
No Ct value	Undetermined	A Ct value was obtained from only one of the 3 PCR targets or the IS900 target was not positive with other targets. This can be indicative of other Mycobacterium or nonspecific reactivity. -A follow up MAP PCR or fecal culture is recommended.
No Ct value	Negative	Indicative of no detection of target nucleic acid.

A Negative/Undetermined result does not rule out the possibility that an animal is infected. With variability of MAP fecal shedding pattern, 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. Repeat testing may be needed to establish the disease status of individual animals.

If an unexpected positive result is obtained on a previously negative animal, it is suggested to proceed in the following manner to confirm the positive result: 1) if time permit, request a Johne's Disease liquid culture (42-56 day incubation, followed by PCR), 2) request retest of the same sample, 3) collect a new sample and resubmit.

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Johne's Disease 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 Johne's Disease Direct 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 *Mycobacterium avium* ssp. *paratuberculosis* detected, no MAP detected by either liquid culture or PCR
***Mycobacterium avium* ssp. *paratuberculosis* detected,** MAP was detected by culture and confirmed by PCR.
Retest Required, is used when inconclusive or undetermined results occur. These can indicate that a different *Mycobacterium* is present that causes a single positive result on the PCR, but not two of the three.

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.
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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.