



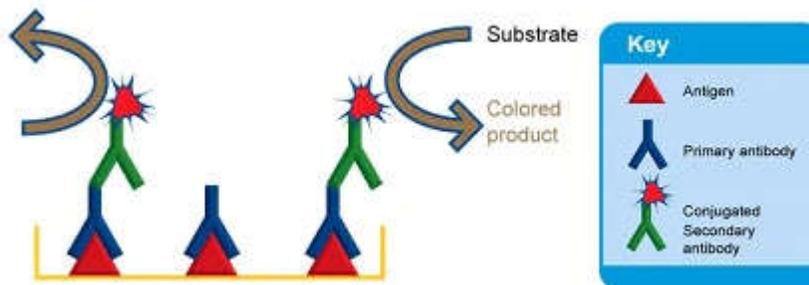
**Wisconsin Veterinary
Diagnostic Laboratory**
UNIVERSITY OF WISCONSIN-MADISON

Johne's Disease Interpretations

Johne's Disease Antibody ELISAs

Valuable diagnostic information can be gained from 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 two kits available in the U.S. for the diagnostic detection of MAP-specific antibodies. The ThermoFisher Prionics PARACHEK™ 2 ELISA (replacing the PARACHEK™ ELISA) and the IDEXX Laboratories, Inc MAP Antibody ELISA. The WVDL is currently providing and is proficiency tested using both kits for the detection of MAP specific antibodies. The Prionics PARACHEK™ 2 ELISA is primarily used, and unless specifically requested, this ELISA is used for routine testing. The IDEXX MAP Antibody ELISA is used as a confirmatory test or when specifically requested by a client. It is important to remember that each kit manufacturer develops a proprietary antigen that is used to bind antibodies that are specific to that MAP antigen. Since IDEXX and Prionics use different MAP antigens, 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, but not the other. 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

Prionics PARACHEK™ 2 ELISA:

The following numerical scoring system is designed to aid in the clinical management of Johne's disease in tested herds using the Prionics PARACHEK™ 2 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 PARACHEK™ 2 ELISA can use bovine, ovine and caprine serum and plasma and bovine milk.



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For serum and plasma samples:

<u>Interpretation</u>	<u>Explanation & Recommendation</u>
0.0 - .15 Negative	Antibodies to <i>M. paratuberculosis</i> were not detected. In <i>M. paratuberculosis</i> 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.
> 0.15 Positive	Antibodies to <i>M. paratuberculosis</i> were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with <i>M. paratuberculosis</i> .

For bovine milk samples:

<u>Interpretation</u>	<u>Explanation & Recommendation</u>
0.0 - .10 Negative	Antibodies to <i>M. paratuberculosis</i> were not detected. In <i>M. paratuberculosis</i> 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.
> 0.10 Positive	Antibodies to <i>M. paratuberculosis</i> were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with <i>M. paratuberculosis</i> .

Note: Occasionally clinically ill cattle with advanced Johne's disease confirmed by a positive fecal culture or PCR test are negative when tested with the ELISA assay. The reason for this is unknown. ELISA results should not replace sound clinical judgment based in part on herd history, experience, herd testing information and use of ancillary diagnostic tests such as fecal culture or PCR. All positive ELISA results are repeated in duplicate prior to being submitted on the final report to the client. Results of the test are reported as S/P ratios, which is the result of the sample divided by the result of the positive control. These S/P values directly relate to the likelihood of an animal being infected and shedding MAP in feces.

The Prionics PARACHEK 2 Mycobacterium paratuberculosis ELISA test kit is in the process of being licensed by the USDA. Kits manufactured under this license will be available in 2016. USDA APHIS VS has authorized use of PARACHEK 2 for export purposes at this time (as of 10/30/2015) until the USDA licensed kits are available. The PARACHEK™ 2 ELISA was first approved by the European Union for use in Europe.

It is recommended that suspect and positive results be confirmed using fecal liquid culture or direct PCR for *Mycobacterium avium* subspecies *paratuberculosis*.



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IDEXX 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 IDEXX 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 IDEXX MAP Antibody ELISA is only used for bovine serum and plasma and milk samples.

For serum and plasma samples:

<u>Interpretation</u>	<u>Explanation & Recommendation</u>
0.0 - .45 Negative	Antibodies to <i>M. paratuberculosis</i> were not detected. In <i>M. paratuberculosis</i> 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.
.45 - .55 Suspect	Cattle with ELISA results in this range are more likely to be <i>M. paratuberculosis</i> 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.
> 0.55 Positive	Antibodies to <i>M. paratuberculosis</i> were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with <i>M. paratuberculosis</i> .

For bovine milk samples:

<u>Interpretation</u>	<u>Explanation & Recommendation</u>
0.0 - .20 Negative	Antibodies to <i>M. paratuberculosis</i> were not detected. In <i>M. paratuberculosis</i> 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.
.20 - .30 Suspect	Cattle with ELISA results in this range are more likely to be <i>M. paratuberculosis</i> 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.
> 0.30 Positive	Antibodies to <i>M. paratuberculosis</i> were detected. A few clinically normal animals with high ELISA values will be fecal culture or PCR negative and may not be infected with <i>M. paratuberculosis</i> .

Note: Occasionally clinically ill cattle with advanced Johne's disease confirmed by a positive fecal culture or PCR test are negative when tested with the ELISA assay. The reason for this is unknown. ELISA results should not



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replace sound clinical judgment based in part on herd history, experience, herd testing information and use of ancillary diagnostic tests such as fecal culture or PCR. All suspect and positive ELISA results are repeated in duplicate prior to being submitted on the final report to the client. Results of the test are reported as S/P ratios, which is the result of the sample divided by the result of the positive control. These S/P values directly relate to the likelihood of an animal being infected and shedding MAP in feces (1).

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

It is recommended that suspect and positive results be confirmed using fecal liquid culture or direct PCR for *Mycobacterium avium* subspecies *paratuberculosis*.

Johne's Direct Fecal PCR:

At the WVDL, we use the Life Sciences Johne's Direct Fecal PCR that detects *Mycobacterium avium* subsp. *paratuberculosis* extracted from fecal samples of cattle, large ruminants, goats and sheep using the ISMap02 gene using the Life Sciences. Fecal samples can be pooled in groups of five. Pools are made at the laboratory so that if a positive is detected, we can test the individual sample. 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.

<u>Ct Value</u>	<u>Explanation & Recommendation</u>
Ct value ≤ 23	Strong positive reaction indicative of an abundance of MAP nucleic acid
Ct value 23.01 to 22	Moderate positive reaction indicative of moderate amounts of MAP nucleic acid
Ct value 33.01 to 37	Weak positive reaction indicative of small amounts of MAP nucleic acid
Ct value 37.01 to 40	Inconclusive. There is low confidence that Ct values in this range can be distinguished from a negative result. A follow up MAP fecal culture test is recommended.
No Ct value	No MAP detected

A "not detected" 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 results 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 (a 42-day test) if time permits followed by IS900 PCR, 2) ask for a retest of the same sample, 3) collect a new sample and resubmit, and lastly, and most expensive, 4) submit a new sample to two laboratories using two different PCRs that have different gene targets (such as HspX, Mav2, ISMAP02, or IS900).



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Johne's Liquid Culture:

At the WVDL, we use the Versa Trek Johne's culture system (Trek Diagnostic Systems Inc.) for the replication and detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) followed by confirmatory PCR for positives, or upon request for all samples, and acid-fast staining for all samples. This is the most sensitive test that we offer, but requires an extensive incubation periods. Fecal samples can be pooled in groups of five. Pools are made at the laboratory so that if a positive is detected, we can test the individual sample. The Trek system uses 2 gram of feces diluted in sterile water, followed by two overnight enrichments in broth with antibiotics. Afterwards, the sample is mixed with proprietary media, as described by the manufacturer, and incubated in capped bottles for 42 days for bovine samples and 56 days for caprine, ovine and other species samples. The Versa Trek System detects pressure changes in the head space of the bottle caused by the growth of MAP and plots those changes on a graph, which determines positivity. All samples are confirmed using acid-fast staining and positive samples, or upon request all samples, are also confirmed using polymerase chain reaction for the *IS900* gene. The results are semiquantitative and listed below for bovine samples only.

Days of Incubation

Explanation & Recommendation

Day: 0-14

+4, Strong positive reaction indicative of an abundance of MAP

Day: 15-28

+3, Moderate positive reaction indicative of moderate amounts of MAP

Day: 29-42

+2, Weak positive reaction indicative of small amounts of MAP

> 42 days, but PCR positive

+1, Very weak positive reaction indicative of a small amount of MAP

> 42 days, and PCR negative

Negative, No MAP detected

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.