To Access Appendix G and all hyperlinks on line, please go to
http://ahdc.vet.cornell.edu/docs/Johnes_Disease_Program_Sampling_and_Testing.pdf

Johne's Disease Program: Sampling and Testing Options

The NYS Department of Agriculture & Markets provides Johne's testing subsidies for all herds in New York State. The Johne's Program in New York State (NYS) is administered through the NYS Cattle Health Assurance Program (NYSCHAP). An additional testing subsidy is provided to herds enrolled in the NYSCHAP Johne's Module. Out-of-State Johne's testing is offered at the full fee; please refer to the Animal Health Diagnostic Center's Test & Fee Schedule listings for specifics on individual tests. Johne's Disease, or Pseudotuberculosis is caused by the bacterium, *Mycobacterium avium* subsp paratuberculosis (MAP). The laboratory participates in an annual proficiency test for Johne's serology, cultures, and PCR tests and is approved to do Johne's testing for the USDA Voluntary Johne's Control Program.

Species	Herd Testing Options *	Individual Animal Testing Options
CATTLE: Testing is not recommended for cattle < 18 mo. of age (see section **B**, below, for assistance with test selection and section **E**, below, for test method discussion)	**Johne's Direct Fecal PCR:** feces Whole herd or subset testing: see Direct Fecal PCR Fact Sheet for Veterinarians **Johne's Culture, Bovine:** feces Whole herd or subset testing. **Johne's Culture, Pooled**: feces With individual PCR follow-up (separate fees apply) **Johne's commercial ELISA:** serum Whole herd testing. Suitable for testing Bos Taurus and Bos indicus breeds.	**Johne's Direct Fecal PCR:** feces See Direct Fecal PCR Fact Sheet for Veterinarians. No pooled PCR testing is available. Pooling of samples prior to submission should be discouraged. **Johne's Culture, Bovine:** feces/tissues **Johne's AGID, Bovine:** serum For testing clinical suspects. Also recommended for Cervidae in addition to JAGIDH **Johne's commercial ELISA:** serum Not intended to be interpreted as an individual cow test. If utilized individually then PCR confirmation recommended on feces. It should also be noted that there is a subset of animals that may be negative on this ELISA but still shedding MAP **Johne's CF:** serum For export / regulatory tests **ONLY**
ALL Other Ruminants: Sheep, Goats, Deer, Camelids, Exotics (see section **C**, below, for assistance with test selection)	**Johne's Culture, Non-Bovine:** feces (Note: sheep see section **C** below). Whole herd or subset testing. No pooled testing available. **Johne's commercial ELISA:** serum (sheep, goats). Contact lab for other species or use Johne's AGID **Johne's AGID** (JAGIDH): serum (Goats, deer, camelids, exotics). Not offered for sheep. We recommend running the Johne's AGID, Bovine in addition to this test for Cervidae.	**Johne's Culture, Non-Bovine:** feces or tissues (see section **C** below) **Johne's commercial ELISA:** serum (sheep, goats and other exotic ruminants) Please contact lab for more information. **Johne's AGID:** serum (goats, deer, camelids, exotics). Not available for sheep. **Johne's CF:** serum For export / regulatory testing **ONLY**

***** See NYSCHAP Website/Johne's Module Sect 2.1 for more on herd testing strategies

Animal Health Diagnostic Center (AHDC)

Contacts for the Johne's Program

For all herd sampling, please contact the AHDC to schedule sample submissions.
Monica Carey can be reached at 607-253-4473.
For testing consultation, contact Dr. Belinda Thompson by e-mail at BT42@cornell.edu or call 607-253-3908
or Dr. Erin Goodrich by email at elg25@cornell.edu or call 607-253-3972

A. Sampling for Johne's Testing

- **All veterinarians planning to submit more than 75 herd based fecal samples at one time, must contact the lab in advance by calling Monica Carey at 607-253-4473.**
- The animal's sample number (on the submission form, it is the column to the left of the animal ID column) **must** be written on all blood tubes and fecal containers with a **waterproof marker**.
- Circle the sample number on the container to distinguish it from the animal's ID. Labeling the tube with the animal ID is also very helpful in keeping samples properly identified.
- On the accession form, please specify the **exact name** of the testing option from the table above, as more than one test can be performed on a single sample type.
- Use same animal ID from test to test to track progress of animals.
- For proper test interpretation, a brief Johne's history must be provided with date of birth and species tested.
- For forms and information, call Monica at 607-253-4473. For sample containers and mailers, contact shipping at 607-253-3935 or (https://ahdc.vet.cornell.edu/Supplies/index.cfm. We recommend use of the plastic screw-cap fecal container available for fecal samples from our shipping department.

Direct Fecal PCR and Fecal Cultures

- Please call Monica Carey at 607-253-4473 to schedule testing for greater than 75 samples (except for clinical animals). If the complete herd is being tested, schedule with the laboratory at least ***four*** weeks in advance of submission, if possible.
- Use a clean plastic sleeve to collect each sample to preclude cross-contamination. Feces **MUST** be transferred to an appropriate container. Containers must be clean. Containers should be wide mouthed, unbreakable, sterile screw-cap plastic jars that can withstand internal pressures associated with shipping and bacterial fermentation. Recommended fecal containers may be purchased from the laboratory (https://ahdc.vet.cornell.edu/Supplies/index.cfm; 607-253-3935; ahdlshipping@cornell.edu)
- Fill container ½ full with feces taken directly from rectum and seal tightly. Samples that are liquid should be placed in the fecal cup and placed individually in a zipper lock bag. At least 15 grams (3 tsp) of feces is needed. **Containers that are over half-full or have loose lids can leak, cause cross-contamination, and may be rejected at the laboratory. Please do not use plastic sleeves or gloves as the primary container.**
- Containers should be numbered sequentially to match the order of submission as listed on the submission form. Animal ID can also be written on the container but that is not necessary if the animal ID is clearly provided in the proper sequence on the submission form, and the sample number is correct and legible. Please be sure to use an indelible (permanent) marker.
- Put no more than 25 containers in a leak-proof zipper-lock plastic bag; then place one or more bags in an insulated shipping container with cold packs. Include absorbent material inside each zipper-lock bag. Ship with UN 3373, Biological Substance, Category B Label on outside of box.

Occasionally fungal and bacterial contaminants overgrow fecal samples. Contamination might be linked to feeds or environment of particular farms. To minimize contamination, fecal samples should be taken and shipped promptly (with cold packs). Do not freeze samples **unless** they cannot be submitted within 48 hours of collection, then they should be frozen at -20°C (chest freezer) to reduce mold over-growth. Avoid storing in freezer portion of a refrigerator.

- For Johne's pooled culture testing, individual animal samples must be submitted as above. **Do not pool samples prior to submission.** Pooling will be performed at the laboratory.
- Unscheduled samples may be delayed. Please call Monica Carey at 607-253-4473 to schedule testing of more than 75 samples.
- Samples must be properly taken, submitted, and shipped. A $50/hr fee to cover additional laboratory handling of improperly collected, labelled or shipped samples may be assessed

Johnes's Environmental Cultures

- See section D, below, for a complete discussion on environmental sampling

Johne's commercial ELISA

- Test is performed on serum samples. Please submit 2 mls of serum.
- Johne's commercial ELISA results will be available 2-7 days after submission of serum samples.
- Confirmation of positive ELISA results by Direct Fecal PCR on feces is strongly recommended. Follow ALL submission criteria for fecal samples as stated above.

Johne's AGID Serology

- Test is performed on serum samples. Please submit 1-2 mls of serum.
- For Johne's AGID Herd testing, (deer, camelids & exotics only), call Monica at 607-253-4473 to schedule herds/flocks over 20 animals.

Other Serological Tests

- CF is only run when required for export or regulatory purposes.

B. Rationale for Selection of Tests For Cattle

The recommended test or test combinations depend upon the specific farm situation. Review the herd situation thoroughly with the herd managers. Determine the probable extent and impact of Johne's disease in the herd, farm goals, practices predisposing to spread of infection, and the client's goal and resources for Johne's control. Veterinarians may call 607-253-3908 or 607-253-3892 to arrange consultation with a veterinary support veterinarian on an optimal test regimen. Description of testing strategies can also be found on the NYSCHAP web site in the Johne's Module materials, Section 2. The expected herd prevalence of infection plays an important role in selection and interpretation of tests for Johne's disease and in the actions taken based upon test results.

About 90% or more of long-standing infections with clinical disease are detectable by testing. Early infections, however, do not necessarily stimulate antibody production, and shedding of organisms may be intermittent; thus, fewer than 45% of such animals are detectable by culture (< 20% by serology) at a single point in time. Repeated testing over time enhances detection of subclinically infected animals and will increase assurance that a herd is low risk for *Mycobacterium avium* subsp paratuberculosis (MAP) infection.

Our serological test for screening for MAP infection in non-clinical cattle is the commercial ELISA. While the average specificity of serological tests range from 97 to > 99%, false-positive results can occur from exposure to other cross-reacting environmental mycobacteria. For this reason, serological testing is considered a presumptive test at the herd or individual animal level. Confirmation of seroreactors by PCR or culture is strongly recommended. It should also be noted that there is a subset of animals that may be negative on this ELISA but still shedding MAP.

Selection of Tests for Bovine Herd Testing

Testing is most useful at the herd —and not the individual animal —level. Johne's direct fecal PCR, Johne's culture, pooled fecal culture, and the commercial ELISA tests can be used in various combinations, with the most information for detecting MAP infection coming at the greatest cost. Herd testing options, from highest to lowest quality of information and cost, are as follows:

1. Johne's direct fecal PCR plus Johne's commercial ELISA on all animals—the most aggressive and costly method. May be useful in herds where the goal is complete eradication and removal of infected non-shedders is desired.
2. Only Johne's direct fecal PCR on all animals
3. Johne's pooled fecal culture (pools of 5 created in the lab from individual samples submitted); only economical in low prevalence herds.
4. Johne's commercial ELISA on all animals first, with Johne's direct fecal PCR follow-up of animals with positive values. In herds of unknown history, we recommend using this strategy to initially screen a herd for Johne's infection. It should be noted that there is a subset of animals that may be negative on the ELISA but still shedding MAP.
5. Only Johne's commercial ELISA on all animals for estimation of herd prevalence of Johne's infection (may not be accurate in some herds with cross-reacting bacteria);

Testing of Individual Cattle

As described above, no single test will detect all infections because animals respond differently at different stages of infection. Tests generally work best in individual animals with advanced infection showing clinical signs; 85-90% of such cases have antibody and close to 100% will be shedding high numbers of MAP. However, 10-15% of clinical cases do not have an antibody response. Animals with early, subclinical infection intermittently shed organisms in their feces and < 20% have an antibody response at this stage of infection. **Therefore, test results for individual**

animals must be interpreted with caution. Negative test results do not necessarily mean that an animal is not infected. As with herd testing, repeated testing of an individual animal over time provides more assurance of a lower risk status for Johne's infection.

- **High sensitivity, rapid turnaround, and reasonable fees make the direct fecal PCR the test of choice for clinical suspects**.
- If feces cannot be obtained, the **Johne's AGID, Bovine** would be the recommended serologic test for clinical suspects. (see section E, below, for more information on the AGID)
- Histopathology: A presumptive diagnosis of Johne's disease can be made with compatible histopathology, including acid-fast staining of tissues. Feces or colon contents can be tested by direct fecal PCR to confirm the diagnosis. Also tissues from the distal ileum, ileocecal junction and associated lymph nodes, and mesenteric lymph nodes can be cultured to confirm the diagnosis.

Export and Regulatory Testing

The commercial ELISA may be an appropriate test. We will run CF tests only when required for export/regulatory purposes.

C. Rationale for Selection of Tests for Sheep, Goats, Deer, Camelids, and Exotic Ruminants

Culture: Strain variants of *Mycobacterium avium* subsp paratuberculosis (MAP), have been identified in sheep, bison, and occasionally in goats. Sheep, goats, farmed cervids, and camelids can also be infected with the bovine strain type. The strain variants are more fastidious than the bovine strain type and require different culture methods and longer incubations periods. Cultures are routinely held for 16 weeks on sheep samples and for 12 weeks on all other non-bovine species. Tissue culture currently appears to be more sensitive than fecal culture; however, advances in fecal culture are being made.

As in cattle, serologic tests for Johne's disease generally detect individuals in later stages of infection. Approximately 10% of clinical animals are seronegative and on average, serology will detect 20-30% of infections; therefore, a negative test does not always indicate freedom from infection in the individual animal or at the herd level. Repeated testing provides a more accurate assessment of infection status of individuals or herds. Cross-reactions can occasionally cause false positive seroreactions. Culture and/or histopathology with tissue culture is considered definitive and is recommended for confirmation in flocks or herds with elevated serology. The AHDC is currently performing this commercial ELISA test for all sheep and goat serum samples. The AGID is still recommended as the serology test to use in goats with clinical signs. For cervids, camelids and other exotic ruminants the AGID will continue to be the default serology test.

Note: A finding of repeat AGID positives in herds with compatible pathology but negative fecal cultures warrants further investigation into the possibility of infection by an unculturable strain variant of MAP.

Selection of Tests for Ruminants other than Cattle

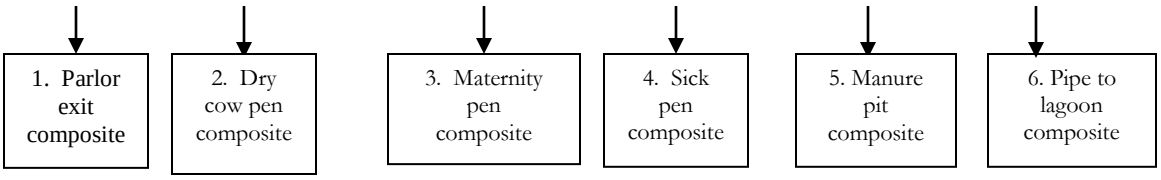
- The **ELISA** test will be used for flock screening of sheep and on clinical suspect sheep. For flock testing, we recommend testing animals 1 year or older.
- The **AGID** test will be used for flock screening in camelids, cervids and other exotic ruminant (see section **E** for more detailed information on AGID testing) and on clinical suspect. For flock testing, we recommend testing animals 1 year or older.
- Fecal culture: Please contact the laboratory to schedule testing prior to submission of cultures for flock testing.
- Histopathology with acid-fast staining of tissues from the distal ileum, the ileocecal junction, and the mesenteric lymph nodes and culture of these tissues are also considered diagnostic.

D. Strategy and Instructions for Environmental Sampling

Sample submission protocols recommended by the Animal Health Diagnostic Center

Dairy *EXAMPLE:* (Adult Herd, >24 months) Flow Chart of Sampling Scheme

Manure Concentration I	**Manure Concentration II**	**Manure Storage**
- Parlor exit/ entrance - Drycow/prefresh pen - Common alleys/gutters	- Maternity pen - Sick pen - End of scraper lanes	- Manure pit - Manure lago



Note: ***Six composite samples are required*** *(as shown in the above example)* for New York State subsidized prices to apply . *Four of the six samples should come from common cow areas (boxes 1 and 2) that represent the entire adult herd present at the point in time of testing. (see point 2 below under Dairy Herd Johne's Environmental Testing Strategy).* This six sample strategy has been scientifically validated for detection of MAP infection at the herd level.

[1] Each of the 6 required composite samples should consist of 4 subsamples totaling 20 grams per location. Use a clean container or disposable zip lock bag to mix the composite well and submit ~10 grams (1/2 of a standard fecal cup) to the AHDC. Use a clean glove to sample each different location. Please use a standard Johne's fecal cup filled ½ to a maximum of ¾ full to submit the mixed composite sample.

[2] *If using the federal guidelines for sampling lagoons, please do not submit gauze squares to the laboratory.* Instead, place gauze with feces in mixing container as previously described, mix well and submit feces only in the standard fecal cup. Manure storage samples can also be obtained by using a clean cup fixed to a long pole[1]. Please use safety precautions!

1. Raizman, Wells, et al. *The Distribution of Mycobacterium avium ssp. Paratuberculosis in the Environment Surrounding Minnesota Dairy Farms*. J. Dairy Sci. 87:2959-2966.

Laboratory Paperwork: NYSCHAP form must be used for NYSCHAP herds to receive rebate.

- **Complete sets of six samples are required for NYSCHAP or NYS subsidized testing**
- ID column - Please identify the specific location sampled for each of the six composite samples
- AHDC Accession Form – in species and breed columns – to identify farm operation type state (Bovine) and (beef or dairy) respectively in the species and breed columns. On the NYSCHAP form indicate beef or dairy in the breed column and bovine in the comment column.
- Sample type should be listed as manure (= manure from the adult cow environment not directly from cows).
- Test requested should be Johne's environmental culture.
- Please specify Johne's environmental testing strategy in the history section and indicate if strategy is used for USDA Test Negative Status Program or herd monitoring.

Dairy Herd Johne's Environmental Testing Strategy:

1. **USDA Test Negative Status Program:** Annual environmental cultures can be used as one testing option for Dairy herds to achieve Level 1 or to maintain monitored status of testing levels already achieved. *Samples should include composites representing the entire adult herd including dry cows.* See link at (USDA Johne's Control Program)
2. **NYSCHAP and NY Program Recommendation:** The objective of environmental sampling in this protocol is to screen a herd for *Mycobacterium avium* subsp paratuberculosis (MAP) infection or to monitor MAP infection loads in known infected herds. For monitoring herds of unknown status, a minimum of twice annual sampling ~ 6 months apart is recommended. **Complete sets of six samples are required for NYSCHAP or NYS subsidized testing.** Samples from common cow alleys (including dry cows) should represent a commingled sample of all adult cows in the herd on the day of sampling. Sampling large manure storage areas such as lagoons can be complicated because storage samples may represent a commingled sample (manure and sometimes milk house waste) collected over a longer period of time with dilution effects and declining MAP survival over time. **When possible, sampling from or near common entry pipes delivering fresh manure to the lagoon or tank from the adult barns without milk house waste can reduce some of the dilution and survival issues. For additional examples, see (USDA Johne's Control Program)**

Recommendations for Sampling Beef Herds:

- ***Herd monitoring only:*** *Johne's Environmental Testing is not currently recognized by USDA for the Johne's Test Negative Status Program in beef herds.*
- **Complete sets of six samples are required for NYSCHAP or NYS subsidized testing.**
- Spring and Fall sampling time frames are recommended.

- **Recommended Locations:** Common sites that represent the adult cattle (including bulls) in the herd.
  - Prefresh and Calving pens or paddocks, Sick Pen (if separate)
  - Common lanes or alleys including the chute when the herd is being handled
  - Around congregation points on pasture including:
    - Feed troughs, Hay manger
    - Water troughs
    - Mineral or supplement feeding stations

E. Brief Description of Test Methods Used in the Johne's Program

Johne's Direct Fecal PCR

For more information, please see the Direct Fecal PCR Fact Sheet for Veterinarians
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 MAP 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 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.

Johne's Fecal Culture

The Laboratory uses a liquid media culture system for bovine samples. For non-bovine fecal cultures, a combination of liquid media culture and solid media culture is used. For sheep, cultures set in liquid media are incubated for a maximum of 70 days. Cultures set on solid agar are incubated for 16 weeks. For goats, cervids and camilids, culture in liquid media is incubated 56 days and cultures set on agar are incubated 12 weeks. Both cultures are set simultaneously and positive results reported immediately as detected. Bovine cultures are incubated for 35-42 days and then checked with an acid-fast stain. Bottles with acid-fast organisms detected are sent for PCR confirmation of MAP. The liquid media system offers a final result in approximately 42-49 days for cattle. Semi quantitative results are provided based on days to positive in culture.

Johne's Pooled Fecal Culture

For use in low prevalence herds – Please see (Johne's Pooled Fecal Culture Testing DL 1090**)**
Individual fecal samples should be submitted to the laboratory using standard Johne's fecal culture sampling practices. Please specify Johne's Pooled Fecal Culture on the accession form for test requested. The laboratory routinely pools 5 samples. Leftovers of 3 and 4 samples are pooled. Leftovers of single or 2 samples will be tested individually by PCR. The pooling of samples will be done in the lab and the individual samples will be catalogued and frozen in an Ultra-Low freezer for PCR if part of a positive pool. You will not have to re-submit samples. Individual samples from positive pools will be automatically set for PCR and appropriate charges for the individual PCRs applied to the accession. Consequently, results on positive pool samples may take 49-60 days and this must be considered for timing of testing for optimal use of results. Clinical suspects or high risk samples should not be tested in pools. **As fecal shedding exceeds 10% of the herd and approaches 20%, the tendency will be for most or all of the pools to be positive. Therefore herds with 10% or more expected shedding should avoid pooled cultures.**

Johne's commercial ELISA

The AHDC utilizes a USDA licensed Johne's commercial ELISA test to detect antibodies directed against *Mycobacterium avium* ssp. *Paratuberculosis* (MAP) in bovine serum. As with all Johne's serology the ELISA is intended to be interpreted primarily at the herd or group level rather than at the individual animal level. The ELISA can be a useful tool to monitor herd prevalence of Johne's disease and to assess the prevalence in source farms for purchased replacements. Potentially, the ELISA could also be used as an adjunct in herds where the goal is complete eradication, and the removal of infected non-shedders is desired.

The results of the Johne's commercial ELISA will be reported out as positive or negative with a numerical corrected optical density value assigned that will allow for comparison of results over time. Since the concern of most farms is the detection of cattle that are shedding MAP, and therefore infectious, it is recommended that this serology test be used in conjunction with antigen detection tests on feces such as direct PCR prior to culling animals. It should also be noted that there is a subset of animals that may be negative on this ELISA but still shedding MAP.

Johne's AGID

Agar gel immunodiffusion (AGID) is another antibody detection methodology routinely employed for detecting antibodies against MAP. Results are reported as positive or negative, based on lines of precipitin forming in an agar matrix between antigen and serum sample wells. Published specificity of AGID tests are 99-100%, as compared to ELISA test specificities from 98.2-99.5%[2,3]. The test sensitivity is expected to be relatively high (>/= 80%) for clinical suspects[2,3], and similar to or less than ELISA assays for subclinical animals (8.0-56%)[4,5]. This supports using AGID tests as the most suitable serological test for clinical suspects in the absence of MAP detection by culture, PCR, or histopathology.

Studies in sheep herds with caseous lymphadenitis, but without paratuberculosis, have shown cross reacting antibodies with *Corynebacterium pseudotuberculosis* for some ELISA tests but not for AGID tests, reducing the specificity of ELISAs to 64%[5]. The AGID may therefore be useful in some control efforts, especially for small ruminants and cervidae, especially where culture or PCR assays are either not available or beyond the economic resources for the herd. Currently reagents are not available for sheep AGID testing.

2. Dubash k,Shulaw WP, Bech-Nielsen S, et al. *Evaluation of an agar gel immunodiffusion test kit for detection of antibodies to Mycobacterium paratuberculosis in sheep.* JAVMA (1996) 208 (3):401.
3. Dubash k, Shulaw WP, Bech-Nielsen S, et al. *Evaluation of an enzyme-linked immunosorbent assay licensed by the USDA for use in cattle for diagnosis of ovine paratuberculosis.* J Vet Diag Invest (1995) 7:347
4. Hope AF, Kluver PF, Jones SL, Condron RJ *Sensitivity and specificity of two serological tests for the detection of ovine paratuberculosis.* Aust Vet J (2000) 78 (12): 850-6.
5. Robbe-Austerman S, et al. *Sensitivity and specificity of the agar-gel-immunodiffusion test, ELISA and the skin test for detection of paratuberculosis in the United States Midwest sheep populations.* Vet Res 37 (2006): 553-64.

Sale Testing

See comments for individual animal testing. Current testing provides limited accuracy in defining an individual animal's Johne's infection status.