

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 (see the colored pages in this manual) for specifics on individual tests. The laboratory participates in an annual proficiency test for Johne's serology and cultures and is approved to do Johne's testing by USDA.

Species	Herd Testing Options	Individual Animal Testing Options
CATTLE: Testing is not recommended for cattle < 18 mo. of age (see section B. on the next page for assistance with test selection)	1) Johne's Kinetic ELISA (KELA), Herd: Fecal confirmation recommended on animals with ELISA values > 40 (handled as separate tests)	6) Johne's Kinetic ELISA (KELA): culture confirmation recommended
	2) Johne's Herd Pooled Fecal Culture, with individual culture follow-up (separate fees apply)	7) Johne's Culture: feces/tissues
	3) Johne's Culture (Fecal) Herd: whole herd or subset testing	8) Johne's AGID, RJT: commercial kit, bovine only
	See NYSCHAP Website/Johne's Module _sect 2.1 http://nyschap.vet.cornell.edu/ for more on herd testing strategies	9) Johne's CF, Licensed ELISA kit: for export / regulatory tests
ALL Other Ruminants: Sheep, Goats, Deer, Camelids, Exotic (see section C. on the next page for assistance with test selection)	4) Johne's Culture (Fecal), Herd: (Note: sheep see section C. on next page)	10) Johne's Culture: feces or tissues (see section C.)
	5) Johne's AGID, Herd screening: in-house	11) Johne's AGID: in-house
		12) Johne's CF: only for export or regulatory testing

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 607-266-8965 or call 607-253-3931.
 For testing consultation, contact Dr. Belinda Thompson by e-mail at BT42@cornell.edu or call 607-253-3908.

A. Sampling for Johne's Testing

- 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.
- 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 607-266-8965 or call 607-253-3931. For sample containers and mailers, contact shipping at 607-253-3935. We recommend use of the plastic 1 oz screw-cap fecal container available for fecal samples from our shipping department.

Fecal culture

- If the complete herd is being tested, schedule with the laboratory at least **four** weeks in advance of submission.
- For submissions with 25 or more samples, or to schedule regular herd samples, call 2-4 weeks in advance.
- Use a clean plastic sleeve for each sample to preclude cross-contamination. Containers must be clean.
- Fill container $\frac{1}{2}$ full with manure taken directly from rectum and **seal tightly**. Samples that are liquid should be bagged individually. **Containers that are over half-full or have loose lids can leak, cause cross-contamination, and may be rejected at the laboratory.**

- Put no more than 25 containers in a sturdy plastic bag; then place one or more bags in shipping container.
- Occasionally fungal and bacterial contaminants overgrow Johne's cultures. Contamination might be linked to feeds or environment of particular farms. To minimize contamination, fecal samples should be taken and shipped promptly (with cool packs). **Do not freeze samples.**
- The Laboratory processes fecals for Johne's culture on Monday through Wednesday. Unscheduled samples or samples arriving after Wednesday of each week will be frozen and set in the order that they are received, starting the following Monday. Please keep these considerations in mind when calculating expected turn-around time and allow a 1-2 week buffer. Call Monica at 607-266-8965 with questions.

Serology

- For Johne's AGID Herd testing, call Monica at 607-266-8965 to schedule herds/flocks over 20 animals.

Johne's Kinetic ELISA (KELA) with Johne's Culture (Fecal) follow-up

- Johne's Kinetic ELISA (KELA) results will be available 2-7 days after submission of serum samples.
- Confirmation of elevated KELA values (> 40) by culture is strongly recommended.
- Follow ALL submission criteria for fecal samples as stated above.

Other Serological Tests

- CF is only run when required for export or regulatory purposes.
- USDA-licensed Johne's ELISA antibody tests are only run for export if required by the importing country or by arrangement with the laboratory.

B. Rationale for Selection of Tests For Cattle

The recommended test or test combinations depend upon the specific farm situation. Review herd situation thoroughly with the client. 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. Then call 607-253-3931 or 607-266-8965 to arrange consultation with a laboratory field 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 (<http://nyschap.vet.cornell.edu/>, section 2). The expected herd prevalence of infection plays an important role in selection and interpretation of tests for Johne's and in the actions taken on test results.

About 90% or more of long-standing infections with clinical disease are detectable by testing. Early infections, however, do not stimulate antibody production, and shedding of organisms is 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 Johne's infection. Fecal shedding typically precedes an antibody response so culture is more sensitive than antibody screening; however, in higher prevalence herds with more advanced infection, antibody screening is more economical than fecal testing.

Kinetic ELISA (KELA) is more sensitive than AGID and more accurate than CF. So Kinetic ELISA (KELA) is our preferred serological test for screening for Johne's in cattle. 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 culture is strongly recommended.

Selection of Tests for Bovine Herd Testing

Testing is most useful at the herd—and not the individual animal—level. Johne's Culture (Fecal) on Herds, Pooled fecal culture, and Kinetic ELISA (KELA) tests can be used in various combinations, with the most information for detecting Johne's coming at the greatest cost. Herd testing options, from highest to lowest quality of information and cost, are as follows:

1. Johne's Culture (Fecal), Herd plus Johne's Kinetic ELISA (KELA) on all animals—the most aggressive and costly method.
2. Only Johne's Culture (Fecal), Herd 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 Kinetic ELISA (KELA) on all animals first, with Johne's Culture (Fecal) follow-up of animals with elevated Kinetic ELISA (KELA) values (> 40). From 0 to 50% of a herd may have elevated Kinetic ELISA (KELA) values depending on the severity of infection. In herds of unknown history, we recommend using this strategy to initially screen a herd for Johne's infection.
5. Only Johne's Kinetic ELISA (KELA) on all animals (may not be accurate in some herds with cross-reacting bacteria); fecal culture confirmation of seroreactors is always recommended.

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 *M. paratuberculosis*. 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.

- To confirm a clinical diagnosis, use both Johne's Kinetic ELISA (KELA) and Johne's Culture (see table).
- Histopathology: A presumptive diagnosis of Johne's can be made with compatible histopathology with acid-fast staining of tissues. Tissues from the distal ileum, ileocecal junction and associated lymph nodes, and mesenteric lymph nodes are useful to detect most cases. A definitive diagnosis can be made in most cases with culture of the aforementioned tissues.

Export and Regulatory Testing

We will run CF or licensed, commercial ELISA antibody 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 *M. a. paratuberculosis*, 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 incubation periods. Cultures are routinely held for 8-12 weeks for species other than cattle. Tissue culture currently appears to be more sensitive than fecal culture; however, advances in fecal culture are being made. We recommend that the laboratory be contacted (607-266-8965) prior to submission of samples for culture to review the current status of the culture system for detection of strain variants. Pooled culture is being evaluated as a herd-screening tool.

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. Recently, one commercial cattle Johne's ELISA was validated and licensed for use in sheep and goats with different cutoffs (available by referral). In our experience, AGID offers higher sensitivity in clinical cases and higher specificity and equivalent sensitivity in subclinical cases. AGID will be the default Johne's serology test offered for these species.

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 *M. a. paratuberculosis*.

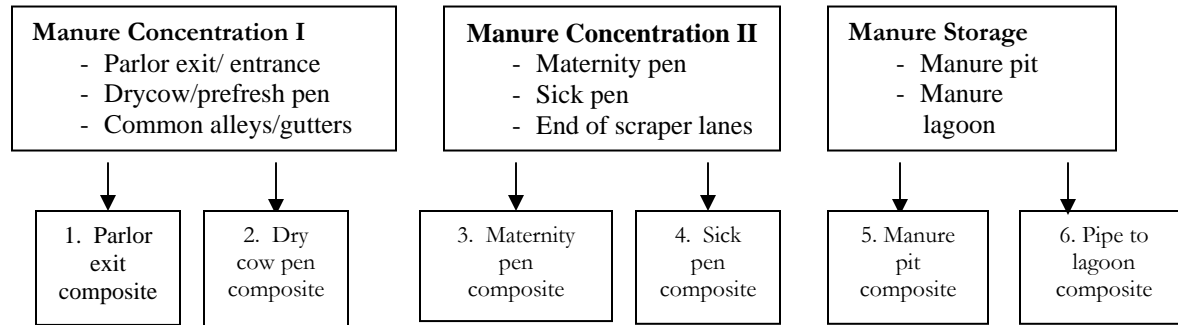
Selection of Tests for Ruminants other than Cattle

- The **AGID** test will be used for flock screening (see table on the first page of this Appendix) and on Clinical suspects unless other arrangements are made with the laboratory. For flock testing, we recommend testing animals 1 year or older.
- The Johne's Culture (Fecal), Herd test is being evaluated in sheep. Contact the laboratory for current status of culture prior to submission of cultures for flock testing. Individual fecal culture on clinical sheep may be useful to determine if bovine strains are present. Johne's Fecal Culture is useful in goats, camelids, and farmed and exotic ruminants because the bovine strain is the major type found. Request Johne's Culture for individual animals, or Johne's Culture (Fecal) Herd for herd screening.
- 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



Note: Six composite samples are required (as shown in the above example). 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 3 - NY Program recommendations).

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 1/2 to a maximum of 3/4 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. Please use safety precautions!

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

- ID column - Please identify the specific location sampled in each composite.
- 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 culture.
- Please specify Johne's environmental testing strategy in the history section and indicate if strategy is used for Test Negative Status Program or herd monitoring.

Dairy Herd Johne's Environmental Testing Strategy:

1. **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 National Johne's Program Standard for full details).
2. **NY - Monitoring in infected herds or herds of unknown status:** Environmental testing is one of several testing options used to monitor Johne's (*M. a. paratuberculosis* or *Map*) loads in a herd or group of cows. For monitoring herds of unknown status, a minimum of twice annual sampling ~ 6 months apart is recommended.
3. **NY Program Recommendation:** The objective of environmental sampling in this protocol is to screen a herd for Johne's infection or to monitor *M a paratuberculosis* infection loads in known infected herds. 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 *M. a. paratuberculosis* survival over time. **When possible, sampling from or near common entry pipes delivering fresh manure from the adult barns without milk house waste can reduce some of the dilution and survival issues. For additional examples, see National guidelines.**

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 in beef herds.*
- Spring and Fall Sampling 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

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

Johne's Fecal Culture

The Laboratory uses a liquid media culture system. 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 *M. a. paratuberculosis*. The liquid media system offers a final result in approximately 42-49 days for cattle. Semiquantitative results are provided based on days to positive in culture.

Johne's Pooled Fecal Culture

for use in low prevalence herds – complete Fact Sheet available

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. Leftover single samples will be tested individually, and 2-4 samples will be included in the final pool. 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 re-culture if part of a positive pool. You will not have to re-submit samples. Consequently, results on positive pool samples may take 80 to 95 days due to the re-culture, 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.

Johne's Kinetic ELISA

The Cornell Johne's KELA ELISA (kELISA) was developed as an in-house Johne's ELISA in 1990 and has been the primary ELISA used for Johne's in the New York program ever since. The Johne's kELISA is intended primarily as a herd or group screening test to be used in conjunction with fecal culture of individuals with elevated values. The Johne's kELISA uses a multiple cut-off interpretation system to increase information gained from the test and more accurately reflect the biology of *M. a. paratuberculosis* infection. The kELISA result is a normalized slope value, a continuous number generally from 0 to 250 that correlates with the amount of antibody in the sample. Result values are reasonably comparable from test to test in the same sample or animal, within a farm, and to a reasonable extent between farms. However, for unknown reasons, ELISA performance can vary from farm to farm. Four cut-offs define four categories, which represent increasing probability or risk that an individual animal is infected.

KELA ELISA units of:	0-39 =	Lowest risk of infection (background activity)
	40-54 =	Moderate risk of infection
	55-164 =	Moderately high risk of infection
	> 164 =	High risk of infection

Within the herd or group, the distribution of KELA values aid in assessing the probability or degree of infection in the group. For information on sensitivity and specificity, the Cornell Johne's KELA ELISA (kELISA) is described in more detail on the NYSCHAP website in the Johne's Module materials (<http://nyschap.vet.cornell.edu/>, section 2).

Sale Testing

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