United States Department of Agriculture

Animal and Plant Health Inspection Service

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Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

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Contents

Introduction

Title 9, *Code of Federal Regulations* (9 CFR), part 71, contains general provisions for the interstate transportation of animals, poultry, and animal products, while part 80 pertains specifically to the interstate movement of domestic animals that are positive to an official test for Johne's disease (JD).

These regulations provide that cattle, sheep, goats, and other domestic animals that are positive to an official test for JD may generally be moved interstate only to a recognized slaughtering establishment or to an approved livestock facility for sale to such an establishment. The animals must bear an official eartag and be shipped with an owner-shipper statement.

Supplementing the regulations are the Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program (VBJDCP) that outline the minimal national standards of the program, providing specifics on administration of the program, program elements and procedures, and laboratory procedures. This document describes the cooperative VBJDCP to be administered by the State and supported by industry and the Federal Government. This publication is intended as a working document that will change as the program develops.

The objective of this program is to provide minimum national standards for the control of JD. The program consists of three basic elements: (1) education, to inform producers about the cost of JD and to provide information about management strategies to prevent, control, and eliminate it; (2) management, to work with producers to establish good management strategies on their farms; and (3) herd testing and classification, to help separate test-positive herds from test-negative herds.

The program has been developed in cooperation with the National Johne's Working Group and the Johne's committee of the United States Animal Health Association, State Veterinarians, and industry representatives. The program has been approved by the USDA's Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS).

The minimum national standards described in this document do not preclude the adoption of more stringent methods and rules by any geographical or political subdivision of the United States with regard to activities within its boundaries; however, regulations dealing with interstate movement must conform to Federal regulations.

I. Definitions and Abbreviations

Accredited veterinarian
A veterinarian approved by the APHIS Administrator in accordance with the provisions of 9 CFR, part 161, to perform functions required by State–Federal–Industry cooperative programs.

Administrator
The Administrator of APHIS or any person authorized to act for the Administrator.

Animal and Plant Health Inspection Service (APHIS)
The Animal and Plant Health Inspection Service of the United States Department of Agriculture.

Animal health official
A full-time employee of the State animal health department or of APHIS who has authority from the State Veterinarian or the Area Veterinarian in Charge to carry out program activities.

Anniversary date
The date on which the designated Johne's disease coordinator gave final approval for initial program participation for a herd.

Approved laboratory
A private, State, Federal, or university laboratory that has passed an annual check test for JD administered by the National Veterinary Services Laboratories (NVSL). The Administrator approves a laboratory to conduct an official JD test only after determining that the laboratory meets the check-test proficiency requirements prescribed by NVSL. Approval continues as long as such check-test proficiency requirements are met annually. All program testing must be done in a laboratory approved by NVSL for the specific test being used in a State's testing program.

Area Veterinarian in Charge (AVIC)
An APHIS veterinarian authorized by the Administrator to supervise and manage the animal health work of APHIS in a specified area of the United States.

CFR
Code of Federal Regulations.

Commingling
Physical contact with susceptible species. For example, all cattle in the same pen, corral, or vehicle or all cattle grazed together on the same area of a property or farm will be considered commingled.

Designated JD coordinator (DJC)
A person who has demonstrated the knowledge and ability to perform the functions required under these program standards and who has been selected for this position by the State animal health official and the AVIC. The VS regional JD epidemiologist and the VS JD headquarters' staff must concur in the selection and appointment of the DJC.

ELISA
Enzyme-linked immunosorbent assay.

Exposure
Contact with known infected animals, contact with the manure or raw milk of infected or exposed animals of susceptible species, or contact with infected herds via contaminated water or feed sources including runoff from neighboring premises.

Herd
A group of animals that has been managed as a separate and discrete unit. This may include two or more geographically separated groups of animals under common ownership or supervision but that have an interchange or movement of animals without regard to health status. The DJC will make the final determination of the herd status of a group of animals.

Herd management plan
A written plan, produced by the Johne's certified veterinarian or animal health official in conjunction with the producer that includes animal husbandry and hygiene practices specific to that herd and that is designed to limit opportunities for exposure to *Mycobacterium avium* subsp*. paratuberculosis*.

Herd member
An animal of any susceptible species that is commingled with the herd.

Infected animal
An animal that has been confirmed by an official JD test to be infected with *Mycobacterium avium* subsp*. paratuberculosis*.

Johne's certified veterinarian
An accredited veterinarian who has completed training approved by the DJC for JD epidemiology and development of herd management plans.

Johne's disease (JD)
An infectious and communicable disease that primarily affects cattle, sheep, goats, and other domestic, exotic, and wild ruminants, also known as paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis*.

Level achievement year
The year a herd obtained its current classification in the herd testing and classification element of the program.

Management herd
A herd for which a risk assessment and herd management plan have been completed that satisfies the requirements of the DJC but has not completed the requirements for the test-negative or test-positive levels.

NCAHP
National Center for Animal Health Programs of USDA, APHIS, VS.

National Veterinary Services Laboratories (NVSL)
APHIS laboratories in Ames, Iowa, and Plum Island, New York.

Official eartag
An identification tag providing unique identification for individual animals. An official eartag must bear the U.S. shield. The design, size, shape, color, and other characteristics of the official eartag will depend on the needs of the users. The official eartag must be tamper-resistant and have a high retention rate in the animal. Official eartags must adhere to one of the following numbering systems:

1. National Uniform Eartagging System.
2. Animal identification number (AIN).
3. Premises-based number system. The premises-based number system combines an official premises identification number (PIN), as defined in this section, with a producer's livestock production numbering system to provide a unique identification number. The PIN and the production number must both appear on the official tag.
4. Any other numbering system approved by the Administrator for the identification of animals in commerce.

Official JD test
An organism detection test approved by the Administrator and conducted in a laboratory approved by the Administrator. A list of currently approved laboratories and the requirements for obtaining approval are available from the Diagnostic Bacteriology Laboratory, NVSL, P.O. Box 844, Ames, Iowa 50010. The Administrator will approve laboratories to conduct an official JD test only after determining that the laboratory meets the check test proficiency requirements prescribed by the NVSL. Approval will continue as long as such check test proficiency requirements are met on an annual basis.

Premises identification number (PIN)
A unique number assigned by a State or Federal animal health authority to a premises that is, in the judgment of the State or Federal animal health authority, a geographically distinct location from other livestock production units. The PIN is associated with an address or legal land description and may be used in conjunction with a producer's own livestock production numbering system to provide a unique identification number for an animal. The PIN may consist of:

1. The State's two-letter postal abbreviation followed by the premises' assigned number; or
2. A seven-character alphanumeric code, with the right-most character being a check digit. The check digit number is based upon the ISO 7064 Mod 36/37 check digit algorithm.

Program
Voluntary Bovine Johne’s Disease Control Program (VBJDCP).

Screening test
A JD test approved by the Administrator for use in the VBJDCP and conducted in an approved laboratory. Screening tests are tools that have been developed to aid in determining the presence or absence of *M. avium* subsp. *paratuberculosis* within a herd. Animals found positive to these tests should be considered suspect unless they show clinical signs of JD (in which case they are considered positive) or they may be confirmed positive or negative by an official JD test.

State
Any of the 50 States, the Commonwealth of Puerto Rico, the Commonwealth of the Northern Mariana Islands, the District of Columbia, and any Territories and possessions of the United States.

State animal health official
The State official responsible for livestock and poultry disease control and eradication programs.

State JD Group
A group of interested persons organized by the State animal health official to assist in the oversight and coordination of the State's Johne's program.

Susceptible species
Domestic and exotic ruminants, such as cattle, bison, sheep, goats, cervids, and camelids, that are capable of natural infection with *M. avium* subsp. *paratuberculosis.*

Test-negative herd
A herd that is enrolled in the program and meets the test-negative component requirements described in this document.

Test-negative level
Level 1, 2, 3, or 4, with each increase indicating a lower probability of JD in the herd.

Test-positive herd
A herd that is enrolled in the program and that meets the test-positive component requirements described in this document.

Test-positive level
Level A, B, C, or D; Level A indicates no prevalence or an extremely low prevalence, and Level D indicates the highest prevalence of JD in the herd.

USDA
United States Department of Agriculture.

VBJDCP
Voluntary Bovine JD Control Program.

Veterinary Services (VS)
The division of APHIS in charge of Federal animal health activities within the United States.

II. Administration

A. DJC

1. General

Each State must have one person to act as its DJC. This person should be selected jointly by the State animal health official and AVIC and be approved by the VS regional JD epidemiologist or Regional Director and the Ruminant Health Programs of VS' National Center for Animal Health Programs (NCAHP). Newly designated person have a 1-year grace period to allow the chosen DJC candidate to meet the education and training requirements. During this period, the candidate is considered to be the acting DJC.

2. Qualifications

Each DJC candidate must:

a. Be a full-time State, Federal, or university veterinarian.

b. Successfully complete a JD epidemiology course that includes on-farm risk assessments and development of herd management plans.

c. Have at least 80 hours of experience in assessing risk, developing herd plans, and classifying JD test-positive animals and herds.

d. Attend refresher courses provided by APHIS' VS at least once every 2 years.

e. Reapply every 2 years to renew designation by submitting a letter of notification through the VS regional JD epidemiologist to the Ruminant Health Programs of VS-NCAHP.

3. Responsibilities

The DJC has the responsibility to:

a. Interpret laboratory test results and classify animals and herds based on the use of official and screening tests.

b. Provide training for State and Federal personnel performing program work.

c. Provide training for Johne's certified veterinarians and develop a mechanism to evaluate and monitor the involvement of the Johne's certified veterinarians.

d. Review the risk assessments and herd management plans submitted by herd owners and Johne's certified veterinarians.

e. Periodically audit the program to determine if it is adequately controlling JD in the State.

f. Assist animal health officials, herd owners, and the herd owner's Johne's certified veterinarian with developing herd management plans as requested or needed.

g. Participate in the program activities as a member of the State JD group.

h. Provide required reports to the area office, VS regional JD epidemiologist and the Johne's staff of VS' NCAHP on the progress of the program.

B. State JD Group

1. General

A JD group or an equivalent must be formed to assist the State in program development, implementation, and review. A representative at the producer level, for either the beef or the dairy industry, is recommended as the chairperson for the group. The DJC must be a member. The group must meet at least once a year.

2. Recommended members

This group should include, but not be limited to:

a. Dairy producers—purebred, commercial, and commodity groups.

b. Beef producers—purebred, commercial, and commodity groups.

c. University and extension faculty.

d. Animal health diagnostic laboratory personnel.

e. Regulatory veterinary medical officers—State, Federal, or field services, or all three.

f. Veterinary practitioners—beef and dairy.

C. Johne's Certified Veterinarians

1. General

States may elect to use the services of private practitioners in addition to State or Federal personnel to assist herd owners in conducting risk assessments and developing herd management plans. States using these veterinarians must determine that they meet the qualifications listed below. The DJC needs to develop a process to closely monitor the herd management plans developed by new Johne's certified veterinarians. For example, the DJC may require the new Johne's certified veterinarian to develop the first one-to-five herd management plans in conjunction with an experienced animal health official.

2. Qualifications

Johne's certified veterinarians must be accredited veterinarians, must have received additional education on JD, and must be able to demonstrate to the DJC that they have the knowledge needed to:

a. Provide appropriate JD risk assessments.

b. Develop approved herd management plans.

c. Understand JD epidemiology, testing, and test interpretation.

d. Understand State and Federal program requirements.

e. Collect and submit fecal, tissue, and blood samples for JD testing.

3. Training

In order to maintain consistent competency levels of practitioners among States, the following curriculum has been outlined.

a. Johne's certified veterinarians must receive training specific to Johne's epidemiology and control. Topics to be included for Johne's certified veterinarian training are:

(1) Overview of Johne's pathology and epidemiology in cattle

(2) Management strategies for preventing JD in dairy and beef herds

(3) Management strategies for controlling JD in dairy and beef herds

(4) Overview of diagnostic tests

(a) Types

(b) Interpretation of results

(c) Strategies for use

(5) How to use handbooks

(a) Information collection

(b) Risk assessment

(c) History, prevalence and management practices

(d) Testing strategy

(e) Management plan development

(f) Tie back JD management to existing management and owner goals

(6) Uniform Program Standards for the Voluntary Bovine Johne's Disease Program

(7) State specific regulations and program standards

b. Johne's certified veterinarians must take a JD refresher course approved by the DJC at least once every 3 years. Topics to be included for Johne's certified veterinarian recertification training are:

(1) Review of Johne's basics

(2) Epidemiology update

(3) Testing and interpretation

(a) New and emerging tests

(b) Best tests for different scenarios

(6) National program review, highlighting any changes

(7) JD economics

(8) Marketing tips

4. Responsibilities

Johne's certified veterinarians have the responsibility to:

a. Provide risk assessments and develop herd management plans that will meet the approval of the DJC when requested by the herd owners.

b. Collect and submit samples according to the requirements set by the DJC.

D. Providing Services to Livestock Owners

Program services may be rendered without expense to the livestock owner; however, owners are responsible for handling their animals. Animal health officials may contract with accredited veterinarians, paraprofessionals, other State and Federal agencies, or the management of privately owned firms, as needed, to assist State and Federal animal health personnel in performing specific program activities.

E. Premises Biosecurity

Persons working on the VBJDCP must use sanitary procedures to minimize the risk of physically transmitting diseases to other premises.

F. Exceptions to Deadlines

Animal health officials must follow deadlines for the herd management plan and testing except when a DJC determines that there are extenuating circumstances. The extenuating circumstances must make it impossible to meet the deadline established for a particular herd. The animal health official will set a new deadline in

consultation with the herd owner, or his or her representative, to accomplish the required activities at the earliest opportunity. The new deadline must be consistent with the principles of JD control and eradication.

G. Administrative Review of a State's VBJDCP Activities and Progress

An administrative review of a State's VBJDCP may involve any or all of the following:

1. VS personnel will conduct ongoing reviews at local and national levels by receiving and examining routine monthly, quarterly, annual, and other statistical and narrative reports that have been prepared and submitted by the State and/or Federal animal health officials in each of the States.

2. The Director, Ruminant Health Programs, NCAHP, VS, will monitor the results of existing State policies and procedures for controlling and eradicating JD by examining factors such as the test/activity and herd management plans.

3. Representatives of the Director, Ruminant Health Programs, NCAHP, VS, will evaluate the information provided by the various States in special reports regarding the authorities and policies for implementing the various minimum standards of the State's VBJDCP.

4. Regional Directors, a regional epidemiologist, and staff personnel of VS will visit various States to observe program procedures and to make general or specific evaluations.

5. Special evaluation teams comprised of several individuals representing State, Federal, and/or industry and academic interests will make visits to designated States in order to make limited or comprehensive reviews of the State's VBJDCP.

III. Program Elements and Procedures

A. Education

1. General

The education element in each State serves as the entry level for producers when participating in the State's voluntary program. The education element must provide producers with basic JD information, management strategies for controlling and eliminating the disease, and information on the various aspects of the State's program. Education can take place through group workshops or one-on-one sessions with the producer's veterinarian. A record of participation should be kept. In the education element of the program, producers should receive information on the topics indicated below.

2. Required topics for education

a. Basic JD information—cause, clinical stages, transmission, etc.

b. Management strategies for:

(1) Manure and waste handling.

(2) Colostrum and milk.

(3) Calves and young stock.

(4) Additions and high-risk animals.

(5) Biosecurity.

(6) Infected animals.

c. Control and testing strategies.

(1) Testing.

(2) Test interpretation.

(3) State program components.

B. Management

1. General

Producers informed about JD may wish to participate in the management element, an intermediate step in the program. This level of participation recognizes producers for implementing approved management practices and plans. At this stage, herd testing is an option available to the producer.

2. Requirements

The following components must be completed to the satisfaction of the DJC

a. Risk assessment

Before developing an individual herd management plan, a Johne's certified veterinarian or an animal health official must conduct a risk assessment to identify aspects of management likely to spread *Mycobacterium avium* subsp. *paratuberculosis* throughout the herd. A copy of the risk assessment must be submitted to the DJC with the herd management plan.

b. Herd management plan

The Johne's certified veterinarian or an animal health official, in conjunction with the herd owner, will develop a herd management plan to prevent the introduction of JD into the herd and to reduce transmission of the disease among animals within the herd. Clinical suspects should be segregated and diagnosed as soon as possible. Culture-positive cattle should be sent to slaughter or rendering. A copy of the herd management plan and risk assessment must be submitted to the DJC for review and final approval.

Guidelines for developing a herd management plan can be reviewed by reading the "Handbook for Veterinarians and Dairy Producers" third edition, 2003; the "Handbook for Veterinarians and Beef Producers" third edition, 2003; and the "How to Do Risk Assessments and Management Plans for Johne's Disease" 2003. Copies of these documents are available through the Ruminant Health Programs, NCAHP, VS. The herd management plan should address management practices that prevent the calves and young stock from becoming infected with *Mycobacterium avium* subsp. *paratuberculosis*. The herd management plan should discuss how each of the categories below will be handled:

(1) Animal identification—All cattle must be individually identified using an identification method approved by the State Johne's group. It is recommended that all animals in participating herds should be individually identified using official eartags. Any previous regulations listed in 9 CFR regarding animal identification with other animal health programs still apply.

(2) Minimum biosecurity measures—These measures should be in place to reduce exposure to manure or milk from cattle of unknown JD status. Care is needed to prevent exposure to other susceptible species (*e.g.*, sheep, goats, farmed deer, camelids, and nonprogram cattle). The herd management plan should include the following biosecurity measures:

(a) Ensure that animals added to the herd come only from low-risk or known-status herds and from known sources (do not purchase from sale yards). Record the source and manage additions as higher risk animals unless you have evidence to the contrary.

(b) Minimize exposure of young stock to manure from adult animals, including other susceptible species. How the exposure is minimized will vary depending on management of the cattle located on the premises.

(c) Minimize exposure of livestock to susceptible animals that are infected or have been exposed to infected animals.

(d) Never feed calves unknown sources of colostrum. Never feed calves unknown sources of milk unless it is pasteurized.

(e) Minimize exposure of feed, water, equipment, and vehicles to manure.

(3) Minimum management practices—For dairy herds, the herd management plan should include the following biosecurity measures:

(a) Keep maternity area clean and dry and separate from other adult animals.

(b) Immediately separate each newborn calf from adult animals.

(c) Provide colostrum from a single identified cow; do not use pooled colostrum.

(d) Feed each calf colostrum from a test-negative or healthy low-risk animal.

(e) Feed calves milk replacer or pasteurized milk. Keep calves and heifers free from exposure to the manure of mature cattle, house by age, and separate from older animals.

(f) Separate clinical suspects from maternity and young stock. Keep records of all clinical suspects.

(4) Minimum management practices—For beef herds, the herd management plan should include the following biosecurity measures:

(a) Keep calving areas as clean and dry as possible.

(b) Minimize the density of cow and calf pairs as much as possible.

(c) Use feeding practices that reduce manure contamination of feed and feeding areas as much as possible.

(d) Provide colostrum from the calf's dam or from another single source that is from a test-negative or healthy low-risk animal.

(e) Raise weaned replacements, separated from older animals.

3. Renewal

   To continue in the program, a herd owner and Johne's certified veterinarian must repeat the risk assessment on years 2, 4, 6, and 10 of program participation. The herd owner and Johne's certified veterinarian must also conduct an annual review of the herd management plan with appropriate changes to the herd management plan. The updated risk assessment and herd management plan must be submitted to the DJC.

C. Herd Testing and Classification

1. General

Herd testing and classification constitute the third program element. The purpose of this element is to recognize producers in the program publicly, if desired, for putting approved management practices and plans into place as well as for separating test-negative herds from test-positive herds. Herds at this stage will continue undergoing herd risk assessments and be subject to herd management plans that were developed under the management element. After initial testing, included herds may participate in either the test-positive or test-negative component of this element according to the test results.

2. Requirements for entry

Herd owners enrolling in the herd testing and classification element must have completed a risk assessment and developed a herd management plan using the guidelines established in the management element.

3. Testing

Initial testing is required to determine the herd's test status, using testing strategies listed in Appendix 2 under Monitoring or Level 1. Herd owners should be encouraged to test statistical subsets (see Table 1—Sample sizes for subset testing in Appendix 1) or greater numbers of cattle when possible.

All samples must be collected by, or under the supervision of, an accredited veterinarian or a State or Federal animal health official. Vaccinated herds are eligible. Vaccinated herds must be tested by an organism-detection test. All samples from herds whose owners are applying for herd classification must be submitted to an approved laboratory.

a. Herds in which a positive test is found will be designated as infected herd. These herds may be placed in the test positive component at the VBJDCP or they may be left in the management level of the program. Herd owners may appeal the herd class infection by appealing the positive animal results.

b. Animals positive for a screening test should be classified as a suspect for *Mycobacterium avium* subsp. *paratuberculosis* infection. It is recommended that suspected animals be confirmed using an official JD test unless *Mycobacterium avium* subsp. *paratuberculosis* has already been confirmed on the premises.

c. Animals positive to an official JD test must be classified as infected and handled in accordance to Title 9 CFR Part 80.

d. Appealing the status of a test-positive animal

(1) For animals found positive to a screening test, a herd owner may elect to confirm the test results as follows:

(a) An official JD test must be submitted by an accredited veterinarian within 45 days of notification of the screening test results.

(b) If the official JD test is negative, the herd may retain its test-negative status, but that animal must be included in the next round of program testing if it remains in the herd.

(c) If the animal that was test-positive to a screening test has left the herd so that no confirmation of the results can be obtained, the DJC should conduct a risk assessment to determine the status of the herd.

(2) To appeal positive results to an official JD test, a herd owner must submit a written statement to the DJC within 30 days of the positive results requesting an appeal and then, at his or her own expense, arrange for an accredited veterinarian to:

(a) Conduct a necropsy of the animal with an official JD test on tissue and histopathology of the ileum and of the mesenteric and ileocecal lymph nodes; or

(b) Conduct a full-thickness biopsy of the ileum and biopsy of the mesenteric or ileocecal lymph nodes with histopathology and an official JD test on tissues and fecal samples taken at the time of biopsy; or

(c) Submit six separate fecal samples from the animal, with samples collected between 30 and 45 days apart. All six samples must be negative for the animal to be considered a test-negative animal.

(d) The herd JD status will be suspended until all testing is completed. Only negative results on all tests will allow the herd to retain a test-negative status.

4. Placement

Herds that test positive must remain in the management element or be enrolled in the test-positive component. Herds that test negative will be eligible to enter the test-negative component.

5. Test-positive component

The purpose of the test-positive component is for the State to assist producers with approved JD control risk assessments and herd management plans in place. States may include assessment levels for herd prevalence. Herds enrolled in this component must use testing protocols approved by the DJC.

a. Requirements for herds in the test-positive component

(1) Application—The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management, identification, testing, and herd addition strategies. This agreement must be renewed every 10 to 14 months from the anniversary date.

(2) Animal identification—All cattle must be individually identified using an identification method approved by the State Johne's group. Any previous regulations listed in 9 CFR regarding animal identification with other animal health programs still apply.

(3) Herd additions—All purchased animals should be from herds with approved herd management plans. Heifers raised off the premises should be raised with the minimum biosecurity and control measures in place.

(4) Testing—The herd owners along with the Johne's certified veterinarian must develop a testing protocol as follows:

(a) All samples must be collected by, or under the supervision of, an accredited veterinarian or a State or Federal animal health official.

(b) Vaccinated animals must be tested using an official JD test.

(c) All samples must be submitted to an approved laboratory.

(d) All animals specified in the test protocol must be tested within 10 to 14 months of the anniversary date or may split the testing in the 12-month period following the anniversary date.

(e) Herds not adhering to the prescribed testing requirements will be placed in the management element.

(f) It is recommended that animals with positive results on an official JD test be identified as infected and restricted to the premises. When infected cattle leave the herd, it is recommended that they go directly to slaughter or rendering. Animals crossing state lines must do so in accordance with Title 9 CFR part 80.

(g) A test-positive animal status may be appealed using the same appeal process described above (Section III, paragraph C, 3, a) for entry into the herd testing and classification element. If an animal is removed from the herd while screening test results are pending, a fecal culture should be collected, submitted, and held at the laboratory. This will allow the owner to appeal the herd level if the animal tests positive to a screening test. The DJC will make the final classification of the animal and herd.

b. Optional—Assessment levels—Test positive

The State's test-positive component may use assessment levels. Herds in a State program may achieve Level A, B, C, or D. Each level classifies a herd based on the apparent prevalence of JD within the herd. The level achievement year should also be indicated. For example, the herd owner who completes Level B testing of the herd in 2001 and elects to remain at Level B in 2002 would have a Level B 2001 status. The level achievement year should be noted because continued monitoring increases confidence that the herd prevalence is within that category. States that use assessment levels must follow the test-positive level requirements listed:

(1) Level A—An annual herd test reveals no screening or official Johne's test-positive animals. Level A can be maintained by testing according to the test strategy protocol found under Monitoring or Level 1 (Appendix 2) every 10 to 14 months of the anniversary date. Herds achieving Level A without a recent history of infection should be encouraged to enter the test-negative program at Level 1.

OR

Negative test results on the whole herd and on bulls over 24 months of age.

(2) Level B—An annual whole-herd test with the addition of bulls over 24 months of age reveals less than 5 percent of animals positive to a screening or official JD test.

(3) Level C—An annual whole-herd test with the addition of bulls over 24 months of age reveals at least 5 percent, but not more than 15 percent, of animals positive to a screening or official JD test.

(4) Level D—Herds should be classified as Level D if either of the following applies:

A positive result from testing according to the test strategy protocol found in Appendix 2 under Monitoring or Level 1;

OR

A whole-herd test with the addition of bulls over 24 months of age reveals more than 15 percent of animals positive to a screening test or official JD test.

c. Renewal and advancement

A herd will remain in the test-positive classification for up to 14 months. For continuation, the herd owner must reapply with a copy of the test results, updated herd management plan, and an agreement to follow the test-positive component requirements. The herd owner can send the DJC a letter of intent to renew or advance if confirmation of screening test results will be received after the 14-month deadline. If the letter of intent is received within 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the five-month deadline must be placed in the management element. Owners of removed herds may reapply after the herds complete the required testing.

6. Test-negative component

The test-negative component must include a herd management plan based on the requirements in the management element. The test-negative component includes a series of levels; owners may improve their herd status (achieve a higher level) by additional testing and biosecurity measures. Each higher level represents a greater probability that the herd is free of JD. However, this does not certify that a herd is free of JD. Herds in the test-negative component may remain at any given level by doing monitoring testing or may advance to a higher status with additional testing. Requirements for herds in the test-negative component include:

a. Application

The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management established in the management element as well as the identification, testing, and herd addition requirements listed below.

b. Herd advancement—Test negative

Test-negative herds may achieve Level 1, 2, 3, or 4. Each higher level represents a greater probability that the herd is free from JD; however, a higher level does not certify that a herd is free of JD. The level achievement year should also be indicated. For example, the herd owner who completes Level 2 testing of the herd in 2001 and elects to remain at Level 2 in 2002 would have a Level 2 2001 status. The level achievement year should be noted because continued monitoring increases confidence the herd is not infected. To advance from one level to the next, a statistical subset must be tested (Appendix 1, Sample Sizes for Statistical Subset Testing), and the herd must meet the level requirements listed below for standard track test-negative component levels:

(1) Standard track—The standard track is designed to allow entry into the program with a minimal investment of funds and gradually increase the producer's investment in the program.

(a) Level 1—The herd owner has developed a herd management plan and has agreed to abide by the requirements of the test-negative component. The herd has had negative test results using a testing strategy equivalent to an 85 percent confidence of having a non-infected herd (see Appendix 2, Current Approved Testing Strategies).

(b) Level 2—Herds have met the requirements for Level 1 and have had negative results using a testing strategy equivalent to a 95 percent confidence of having a noninfected herd (see Appendix 2). Level 2 samples must be collected within 10 to 14 months of the anniversary date.

(c) Level 3—Herds have met the requirements for Level 2 and have had negative results using a testing strategy equivalent to a 98 percent

confidence of having a noninfected herd (see Appendix 2). Level 3 samples must be collected within 10 to 14 months of the anniversary date.

(d) Level 4—Herds have met the requirements for Level 3 and have had negative results using a testing strategy equivalent to a 99 percent confidence of having a noninfected herd (see Appendix 2). Level 4 samples must be collected within 10 to 14 months of the anniversary date.

(2) Herds with previously documented testing equivalent to standard track of the test negative component.

The DJC, after evaluation of the previous testing results and evaluation that herd additions are from low-risk sources, may assign a herd a test negative status of up to Level 3 after the cattle have the required Level 3 testing and the owner signs a declaration that:

"No Johne's disease was known or suspected in the herd during the last 5 years."

(3) Herds with a test-negative status should get an official JD test on all clinical suspects even if they are removed from the herd.

c. Animal identification

All test-eligible animals must be individually identified using official eartags. Any previous regulations listed in 9 CFR regarding animal identification for any other APHIS program still apply.

d. Testing

(1) All samples must be collected by, or under the supervision of, an accredited veterinarian or a State or Federal animal health official.

(2) Vaccinated herds will be eligible for the test-negative component after vaccination has been discontinued. All testing must be done using an official JD test until enough nonvaccinated natural additions qualify for serology testing. The number of nonvaccinated animals will be the sample size required for that size herd for a statistical subset for serology.

(3) All samples must be submitted to an approved laboratory.

(4) Herd removal provisions—If an animal in a test-negative herd tests positive to an official JD test, or if the testing requirements are not followed, the herd must be removed from the test-negative component and placed in the test-positive component or in the management element (unless an appeal is pending). Animals that test positive to a screening test must be confirmed with an official JD test or their status will be removed or suspended.

(5) Appealing the status of a test-positive animal must be done using the same appeal process as for entry into herd classification and testing.

e. Herd additions

(1) Purchased heifers and bulls less than 24 months of age may be added to the herd, provided that:

(a) For Levels 1 and 2:

The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering;

OR

The purchased animal is tested with an official JD test in the next herd test after it reaches 24 months of age. The herd will not lose its status if additions that are test positive and any progeny from females are removed from the herd within 30 days after the positive test and the DJC conducts a followup epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may not need to be removed if the epidemiological study indicates a low risk of exposure. The addition animal reaches herd status and becomes part of the herd after a negative test result on the herd test.

(b) For Levels 3 and 4:

The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering;

OR

The purchased animal is from a herd one level below the status of the purchasing herd and is tested with an official JD test at 12, 18, and 24 months of age and is tested in the next herd test after it reaches 24 months of age. The herd will not lose its status if additions that are test positive and any progeny from females are removed from the herd within 30 days after the positive test and the DJC conducts a follow-up epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may not need to be removed if the epidemiological study indicates a low risk of exposure. The addition animal reaches herd status and becomes part of the herd after a negative test result on the herd test;

OR

If the addition animal is more than one level below the herd's current status, the herd will be demoted one status level. The addition animal must be tested with an official JD test at 12, 18, and 24 months of age and tested in the next herd test after it reaches 24

months of age. The herd will not lose its status if additions that are test positive and any progeny from females are removed from the herd within 30 days after the positive test and the DJC conducts a follow up epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may not need to be removed if the epidemiological study indicates a low risk of exposure. The addition animal reaches herd status and becomes part of the herd after a negative test result on the herd test.

(2) Purchased or replacement animals 24 months of age or older may be added to the herd provided that:

(a) For test-negative Level 1 or 2 herds:

The animal was purchased from a herd with a test-negative level equal to or higher than the herd it is entering;

OR

The animal has a screening test within 30 days before entering the test-negative level herd with negative results, and fecal samples from each addition animal have been collected and submitted between 30 days before and 30 days after arrival for an official JD test, and the herd addition is tested on the next herd test. The herd will not lose its status if additions that are test-positive and any progeny from females are removed from the herd within 30 days after the arrival of the additions and the DJC conducts a follow-up epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may not need to be removed if the epidemiological study indicates a low risk of exposure. The addition's status will remain at its entry level until it has tested negative at the next herd test.

(b) For test-negative Levels 3 and 4 herds:

The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering;

OR

The herd of origin has a test-negative level of one level below. The purchased addition has a screening test with negative results within 30 days before entry into the program herd. Fecal samples from each animal added have been collected and submitted between 30 days before and 30 days after arrival for an official JD test. The herd addition is tested on the next herd test. The herd will not lose its status if additions that are test positive and any progeny from females are removed from the herd within 30 days after arrival, and the DJC conducts a follow-up epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may

not need to be removed if the epidemiological study indicates a low risk of exposure. The addition's status will remain at its entry level until it has tested negative at the next herd test;

OR

If the addition is more than one level below the herd's current status, the herd will be demoted one level, and each addition must have an official test on fecal samples between 30 days before and 30 days after arrival. The herd addition is tested on the next herd test. The herd will not lose its status if additions that are test positive and any progeny from females are removed from the herd within 30 days after the arrival of the additions and the DJC conducts a follow up epidemiological study. If the results from the epidemiological study indicate a high risk of exposure to susceptible animals, the herd will lose its status. Progeny of infected bulls may not need to be removed if the epidemiological study indicates a low risk of exposure. The addition's status will remain at its entry level until it has tested negative at the next herd test.

(3) Once all herd additions have been granted status equal to the herd's current status, the herd may advance in the status program by following the testing protocol for the next level.

(4) Heifers raised off the premises must be raised with the proper bio-security measures in place and with animals at an equal or greater test-negative level.

(5) Test-negative program herds may use semen and embryos from other cattle herds, if the semen used is processed according to Certified Semen Services' standards and the embryos are processed according to International Embryo Transfer Society protocols. Embryo transfer recipient cows must meet herd addition requirements.

(6) Purchasing a test-negative herd

(a) If a test-negative herd is purchased, testing is not required if the purchased cattle remain on the premises. A new certificate will be issued in the new owner's name. The anniversary date will remain the same.

(b) If part or all of a test-negative herd is purchased and the cattle move directly to a premises without cattle, they may retain their test-negative status without testing. A new certificate should be issued. The anniversary date of the new herd is established by the test date of the herd of origin or by a new herd test of the purchased cattle.

(7) Herd level maintenance—Test negative
All levels can be maintained by achieving negative screening test results using a testing strategy for Monitoring or Level 1 (Appendix 2).The level achievement year should continue to be noted because continued monitoring increases confidence the herd is not infected.

(8) Renewal and advancement

A herd will remain at any level for up to 14 months. For continuation of this classification, the herd owner must reapply with a copy of negative test results, an updated herd management plan, and an agreement to follow the test-negative component requirements.

The herd owner can send the DJC a letter of intent to renew or advance if confirmation of screening test results will be received after the 14-month deadline. If the letter of intent is received by 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the extended 5-month deadline must be removed from the test-negative component. Herds removed from the test-negative component may reapply at the test-negative Level 1.

IV. Laboratory Procedures

A. Approved Laboratories

1. General

Official Johne's disease (JD) tests and screening tests used for the VBJDCP may be conducted in a private, university, State, or Federal laboratory. The State animal health official has the authority to decide if any laboratory passing the National Veterinary Services Laboratories (NVSL) check test may participate in the State program. States should have the authority to audit the JD diagnostic laboratories participating in the program periodically. If a laboratory is outside of the State, the State may rely on audits conducted by the animal health officials from the State in which the laboratory is located.

B. NVSL Laboratory Approval Process

1. Two check tests must be passed annually in order for laboratories to be approved to participate in the VBJDCP. The check test procedures are outlined below for official JD tests and screening tests. For further information, please contact NVSL's Diagnostic Bacteriology Laboratory.

    a. A laboratory seeking approval to perform official JD tests must contact NVSL for a test kit of 25 samples. (A valid check test sample from NVSL will be determined by a consensus of at least 70 percent of the laboratories participating in the fecal culture check testing process.)

    b. The laboratory must correctly identify 100 percent of the negative test samples as negative samples.

    c. The laboratory must correctly identify 100 percent of the Too Numerous to Count (TNTC) test samples as positive samples.

    d. The laboratory must correctly identify at least 70 percent of the test samples that were not classified as negative or TNTC as positive samples.

    e. Laboratories will be required to identify at least 50 percent of those samples classified as TNTC by the participating laboratories as TNTC or equivalent samples.

        (1) Laboratories will be requested to report colony forming units (CFU) for each culture tube of solid Herrold's egg yolk media. Each laboratory will also need to provide its criteria for assessing shedding level of *Mycobacterium avium* subsp. *paratuberculosis* in samples categorized as TNTC equivalent.
        (2) A check test fecal sample will be classified as TNTC if at least 50 percent of the laboratories that pass the fecal check test, based on the requirements of items a–d listed above, report the sample as TNTC or equivalent. Samples from laboratories reporting CFU per tube would classify a sample as TNTC if two or more tubes had more than the TNTC equivalent as assessed by the laboratory.
        (3) Laboratories that fail to detect at least 50 percent of the consensus-classified TNTC samples will be notified by NVSL of their proficiency

results with the intent to improve their detection techniques. If a laboratory fails to detect the consensus-classified TNTC samples the next year, they will be required to take additional training at NVSL.

f. The laboratory must use the same procedure and materials during the check test that are used during routine testing.

g. No retest is available within the same fiscal year.

2. Approval process for laboratories performing screening tests (serology tests)

a. A laboratory seeking approval to perform screening tests must contact NVSL for a test kit of 25 samples. (A valid check sample will be determined by NVSL using available licensed ELISA kits.)

b. Laboratories will report the serologic score (sample to positive [S/P] ratio, ELISA score and optical density values) of test samples and kit control.

c. Samples will be divided into extreme and intermediate samples based on consensus median values from all participating laboratories.

d. Extreme samples (strong negative and strong positive) will be graded qualitatively.

e. Laboratories that incorrectly identify any extreme test samples (grade qualitatively) will fail the proficiency test.

(1) Intermediate samples will be graded quantitatively using Z scores. Z scores tell how many standard deviations away from the mean the score resides. A positive Z score indicates that the value is above the mean while a negative Z score indicates that the value is below the mean.

(2) Laboratories must score within three Z scores of the mean value to correctly identify a serology proficiency test sample.

(3) Laboratories with more than two quantitative (Z scored) test results outside the acceptable range will not pass the proficiency test.

(4) A Z score calculation will be used to determine variation of quantitative results. A major advantage of the Z score grading system is that is allows laboratory feedback on whether laboratories are consistently above or below the consensus median value. Calculating absolute values allows a visual representation of consistency, since a laboratory that is not consistently above or below the median will still have a large absolute value Z.

(5) Laboratories with an absolute average Z score of greater than two will be notified that quality control practices should be evaluated. Specific actions could include requiring internal quality control review using NVSL's low positive sera, contacting the kit manufacturer, and evaluating other equipment and procedures.

(6) The laboratory must use the same procedure and materials during the proficiency test that are used during routine testing.

(7) One retest per year is available if a laboratory fails the first time. Any laboratory that fails to pass the retake of the check test will become a nonapproved laboratory until the check test is administered again and passed the following year.

3. Continuing quality assurance

Producers and veterinarians must have confidence in the quality of serology results they receive. To ensure the credibility of the infection status assigned as a result of testing under the guidelines of the national control program, each approved laboratory should run low positive quality control sera purchased from NVSL in order to monitor variations in testing results. NVSL can provide the current protocol for running and submitting the data from the quality controls.

4. Reporting

In order to monitor VBJDCP activity, testing conducted at approved laboratories must be reported to the State animal health official. Data from the laboratory must be reported on a monthly basis. Data should be reported/sorted by State of the herd. The minimum required data for national reporting includes:

a. Number of herds tested by screening tests, sorted by method of detection and species.

b. Number of screening test positive results, sorted by method of detection and species.

c. Number of herds with test positive results by screening tests, sorted by method detection and species.

d. Number of official JD tests submitted and run, sorted by method of detection and species.

e. Number of herds tested by official JD tests, sorted by method of detection and species.

f. Number of official JD tests results that are positive for *Mycobacterium avium* subsp. *paratuberculosis*, sorted by method of detection and species.

g. Number of herds with positive results by official JD test, sorted by method of detection and species.

5. If the required data is not reported on a timely basis, the State animal health official may refuse to allow a laboratory to do State program testing. It is recommended that the State animal health official require that all Johne's test results be reported to the State.

C. Approved Program Tests

1. Official JD tests—An organism detection test approved by the Administrator and conducted in a laboratory approved by the Administrator.

    a. Fecal and tissue culture—Culture is the standard for organism-based tests, although culture methods are not currently standardized. Methods include both solid and liquid culture preparations. Protocols for recommended methods can be obtained from NVSL upon request.

    b. DNA probe—DNA probes can detect the presence of *Mycobacterium avium* subsp. *paratuberculosis* without having to grow it. The test has the advantage that it takes less than 3 days but has the disadvantages of higher cost and the potential of missing low shedders.

    c. Histology of tissue—No check test is available at this time. Microscopic identification of the characteristic pathological changes and of *Mycobacterium avium* subsp. *paratuberculosis* organisms in tissue is a definitive test for JD. Tissue changes and bacteria can be observed in the intestinal lining and in nearby ileal, mesenteric, and ileocecal lymph nodes in infected animals. Sensitivity depends on the stage of disease and the number and type of specimens collected.

2. Screening test—Screening tests are tools that have been developed to aid in determining the presence or absence of *Mycobacterium avium* subsp. *paratuberculosis* within a herd.

    a. USDA approved ELISA—Animals found positive with ELISA tests should be considered suspect until confirmed using an official JD test. ELISA tests are to be used as screening tools or to help make management decisions.

    b. Environmental sampling—Fecal material samples collected in areas where a large proportion of the herd is commingled. Samples are tested using organism detection methods in approved laboratories. The sampling protocol is available in Appendix 2.

    c. Pooled fecal cultures—Fecal samples collected from individual animals that are pooled together in groups of five. Individual samples should be submitted to laboratories approved for fecal pooling and tested by an official JD test. The pooling protocol is available in Appendix 2.

Appendix 1 – Sampling Sizes and Random Selection for Statistical Subset Testing

Sample Sizes

Table 1—Sampling sizes for statistical subset testing

Number of cattle in herd (36 months or older)	Minimum Number of Cattle to Sample (36 months or older)	
	ELISA Testing	Fecal culture
≤ 300	Test all	Test all
301 – 400	Test all	Test all (up to 313)
401 – 500	Test all	324
501 – 600	Test all (up to 531)	332
601 – 700	540	338
701 – 800	547	342
801 – 900	552	345
> 901	580	360

In smaller herds, all cattle 36 months of age and older must be tested. In herds with fewer than 30 animals 36 months of age or older, animals 24 months of age or older must also be tested.

The sample numbers above are based on the following assumptions:

- The cattle to be tested are 36 months of age or older.
- ELISA tests are assumed to have 25 percent sensitivity, and fecal cultures are assumed to have 40 percent sensitivity. (These were consensus estimates of the Herd Status Committee of the National Johne's Working Group of the United States Animal Health Association Committee on Johne's Disease for cows 24 months of age or older with subclinical infections. No changes were made for older populations sampled.)
- ELISA and fecal culture are assumed to have 100 percent test specificity (given follow-up of all ELISA positives with fecal culture).
- The confidence of detecting infection (at least one test-positive cow), if present at a true prevalence of 2 percent, is 95 percent.
- The calculations are based on sampling without replacement.

Random Selection Procedures for Random 30 or Statistical Subsets

Ideally, the veterinarian should get a list of eligible cows and randomly select identification from that list. Then, the veterinarian should collect samples from the animals selected. This works in small herds or in herds where the animals can be easily caught. For a more scientific method, select animals using a random number generator or random number tables.

If the list is made in random order, a systematic sampling can be used on the list (i.e., selecting every **n**th individual from a list [or coming through a chute] after choosing a random number from 1 to **n** as a starting point). Determine **n** by dividing the number in the herd by 30 (or whatever sample size is needed in the statistical subsets).

In large herds, veterinarians should use a more stratified randomization to collect a more representative sample. The veterinarian should determine how many animals are in each group (e.g., high producers, low producers, and dry cows) and compute the percentage of each type of animal. The percentage of samples collected from each group should equal the percentage of each group of animals in the herd. In other words, if 10 percent of the animals in the herd are low producers, then 10 percent of the samples should be collected from low producers. Sampling in each group would be done by simple or systematic sampling.

These will not be perfect randomized sample collections, but they are better than convenience sampling. Unless the producer is suspicious of infection in the herd, targeting "high-risk" animals is not the method to use. Testing high-risk groups or target testing should be reserved for herds in the management or control programs. All test negative status program testing should be as close to random as possible.

Appendix 2 – Approved Testing Strategies

Estimated confidence levels take into account the testing in the current level of the program plus testing that has occurred in previous levels.

Monitoring or Level 1 – 85 percent confidence of having a noninfected herd

A. Negative serum ELISA results on 30 randomly selected animals 36 months of age and older. In herds with fewer than 30 animals 36 months of age or older, animals 24 months of age or older must also be tested;

OR

B. Environmental Sampling—Dairy Farms

From each dairy farm, collect two composite environmental samples tested by an official JD test from each of the following locations on the farm: manure concentration areas (cow housing alleyways or gutters), manure storage areas (lagoons, piles, pits, or manure spreader), and another manure concentration area (sick cow pens or other cow alleyways and travel-ways). A total of six samples should be collected for submission to the diagnostic laboratory.

Each composite environmental sample should contain approximately 20 g of fecal material with bedding or soil from a minimum of four different sites within each sampling location. Collect each sample with a separate disposable latex glove, place in a 95-mL plastic-covered specimen container labeled with the sampling location and herd identifier, and store in a cooler with ice during transport to the laboratory. Be sure to include sufficient manure in the sample when sampling from well-bedded areas such as sick cow pens or loose-housing lots.

In free stall barns, obtain each cow alleyway sample from a minimum of four sites across cow alleyways, collected to represent most of the mature cows (24–36 months of age and older) within the farm. Crossovers (areas around waterers, corners, and ends of scrape lanes) are excellent places to collect well-mingled manure subsamples.

In tie stall barns, obtain samples from all gutters containing manure from mature cows (24–36 months of age and older). Other suitable locations for subsampling are places where the gutter cleaner exits the barn, along the ramp, and in corners.

Sample manure storage lagoons (using personal precautions for safety) from four locations at the perimeter of the lagoon by submerging the sampling container up to 10 cm beneath the water's surface.

Each composite sample from manure piles should be obtained from four different sites up to 10 cm beneath the surface.

Each composite sample from manure pits can be collected using two to three sterile 4 × 4 gauze pads tied to fishing line with fishing weight and soaked at least 10 cm below the manure's surface.

(Modified from Raizman, Wells, et al., 2004. J. Dairy Sci. 87:2959–2966.)

OR

C. Pooled fecal cultures

Test individual fecal samples tested by an official JD test of 50 randomly selected animals, 36 months of age or older, in 10 pools from 5 animals. Submit individual samples to laboratories approved for fecal pooling.

Herds of fewer then 50 adult animals can still use fecal pooling. All animals 24 months or older are to be included until a total of 50 animals has been reached or all 24- month-old animals have been tested. At least 3 pools of 5 (15 animals) should be available to use pooling. If 15 animals are not available, individual samples should be used.

Fecal Pooling procedure:

1. Fecal pooling procedure – to be done in the laboratory – five samples per pool

    a. Homogenization by stirring

        Weigh out 2 grams of each sample to be pooled and place into a sterile 50 ml conical centrifuge tube. Mix samples by stirring with a sterile wooden stick. Vortex samples vigorously for 10 to 15 seconds until the mixture appears homogeneous. Remove 2 g of the resulting mixture for processing and culture by routine method used in laboratory.

    b. Homogenization by stomaching

        Weigh out 2 grams of each sample to be pooled and place into a stomacher bag. Be sure to add the samples to the same corner of the bag to ensure even mixing. Stomach the samples on the highest setting for 2 minutes. Inspect the mixture to determine if it appears homogeneous. Additional stomaching may be required to homogenize the mixture. Remove 2 g of the resulting mixture for processing and culture by routine method used in laboratory.

2. References:

Van Schaik, G. et al. 2003. Pooled fecal culture sampling for *Mycobacterium avium* subsp. p*aratuberculosis* at different herd sizes and prevalence. J Vet Diagn Invest 15:233-241.

Wells, S.J. et al. 2003. Evaluation of bacteriologic culture of individual and pooled fecal samples for detection of *Mycobacterium paratuberculosis* in dairy cattle herds. JAVMA 223:1022-1025.

Wells, S. J. et al. 2002. Evaluation of bacteriologic culture of pooled fecal samples for detection of *Mycobacterium paratuberculosis*. A JVR **63**:1207-1211.

Level 2 – 95 percent confidence of having a noninfected herd

Negative screening tests (ELISA) on a statistical subset (see Appendix 1) of animals 36 months of age or older.

Level 3 – 98 percent confidence of having a noninfected herd

A. Negative official Johne's disease test results on fecal samples on a statistical subset (see Appendix 1, Sampling sizes for statistical subset testing) of herd members 36 months of age or older. Bulls 24 months of age and older must be included in this testing;

Or

B. Pooled fecal samples tested by an official JD test from a statistical subset of animals (see Appendix 1, Sampling sizes for statistical subset testing) 36 months or older may be used to advance a herd from Level 2 of the test-negative program to Level 3 of the test-negative program provided that at least one negative whole herd test has been conducted by screening tests or official Johne's disease test on individual samples. Individual samples must be submitted to the laboratories approved for fecal pooling. Each pool will contain no more than five individual animals and the pooling of samples will be done according to the pooling methods documented for Level 1 of the test negative component at the VBJDCP. Bulls 24 months of age or older must be included in this testing as well.

In addition to the pooled fecal cultures taken from the statistical subset of animals, six composite environmental samples also must be taken according to the protocol described for dairy herds for entrance to Level 1. For beef herds, environmental samples should be taken from areas around feed bunks, waterers and other animal collection points.

In herds with less than the required statistical subset, all animals 24 months of age or older are to be included until the required number of animals has been reached or all animals 24 months of age or older have been tested. At least 3 pools of 5 (15 animals) should be available to use pooling. If 15 animals are not available, individual samples should be used.

Any pool of samples found positive for *Mycobacterium avium* subsp. *paratuberculosis* will cause the herd to be classified as an infected herd. Individual animals included in the positive pool will be considered suspect until individual fecal samples from all animals in the pool are tested using an official Johne's disease test. Those animals with a negative test result on the individual samples will be classified as test negative and animals found with a positive test result will be classified as infected. In the case that a herd has a positive pool test result but all individual animals are tested negative for the presence of *Mycobacterium avium* subsp. *paratuberculosis* on the individual fecal samples, the herd shall remain classified as infected. If the herd owner elects to challenge the test results, they must do so by the appeal process for an official Johne's disease test (see Part III, paragraph C, 3 d) for each animal included in the positive pooled samples. Individual animals classified as infected are subject to reporting and identification and movement restrictions in accordance with Title 9 CFR part 80.

Level 4 – 99 percent confidence of having a noninfected herd

Negative serum ELISA test results on fecal samples on a statistical subset (see Appendix 1, Sampling sizes for statistical subset testing) of herd members 36 months of age or older. Bulls 24 months of age and older must be included in this testing.

Appendix 3 – **Johne's Certified Veterinarian Training Guidelines**

I. Initial Certification Training

A. Johne's Overview

1. Description (strains and survival).

2. Pathogenesis (host susceptibility, stages of disease, differential diagnosis).

3. Transmission pathways (fecal/oral, colostrum/milk, prepartum, saliva, artificial insemination, embryo transfer (E.T.)).

4. Pass-through events.

B. Disease Prevention Management

1. Directed at enhancing biosecurity

2. Include general principles and point out differences between dairy and beef herd management practices.

3. Prevent entrance of *Mycobacterium avium* subsp. *paratuberculosis*

4. Risk from herd additions and replacements (include E.T. recipients and herd bulls)

5. Risk from environment (vehicles, water, feed, etc.)

C. Disease Control Management

1. Directed at reducing risk for pathogens.

2. Include general principles and point out differences between dairy and beef herd management practices to break the infection cycle, prevent ingestion, prevent spread, and decrease pathogen load in the environment.

3. Cover manure management in specific animal environments such as newborns, suckling and weaned calves, yearling and bred heifers and mature animals.

4. Provide information on colostrum and milk management with emphasis on pooled versus individual feeding, effect of feeding nonpasteurized hospital or waste milk, and methods of on-site pasteurization.

5. Include strategies for managing infected animals through animal identification, removal/separation management, and provide information on the importance of protecting susceptible young stock and reducing the environmental pathogen load.

6. Provide ideas for the management of replacements and/or additions including biosecurity of heifers raised off-site, knowing the risk of the source herd of additions and strategies to buy from low-risk sources.

7. Development of a risk management plan based on assessment

D. Overview of Diagnostic Tests

1. Types of tests being used
   a. ELISA
   b. Fecal culture
   c. AGID
   d. DNA probe
   e. Biopsy
   f. Gamma interferon

2. Interpretation of test results
   a. Sensitivity and specificity
   b. Likelihood ratio
   c. Predictive values
   d. Interpretation at the herd level
   e. Interpretation at the individual animal level

3. Discuss differences in strategies for testing to determine herd prevalence, enhancing control, measure herd progress, purchasing herd additions, and participating in the VBJDCP.

E. Overview of Risk Assessments and Management Plans

1. The information in the handbooks is the cornerstone for doing risk assessments and developing management plans ("Handbook for Veterinarians and Dairy Producers" and "Handbook for Veterinarians and Beef Producers").

2. Each topic should be covered in enough detail to ensure that veterinarians are able to collect required data, perform risk assessments and complete management plans with their clients.

3. The instructional handbook, "How to Do Risk Assessments and Management Plans for Johne's Disease", is recommended as a guide for specific information details.

F. Overview of Uniform Program Standards for the Voluntary Bovine Johne's Disease Program

1. Responsibilities of certified veterinarians in the Johne's Program.

2. Explanation of the requirements for the Management element and Herd testing and classification element on the Voluntary Bovine Johne's Disease Control Program.

G. Overview of Specific State Regulations and Program Standards.

H. Model Time Allotments for the Actual Training Sessions (6 – 8 Hours)

1. Topics to be included are:
   a. Overview of Johne's pathology and epidemiology in cattle (1 hour)
   b. Management strategies for preventing JD in dairy and beef herds (30 minutes)
   c. Management strategies for controlling JD in dairy and beef herds (30 minutes – 1 hour)
   d. Overview of diagnostic tests (1 – 2 hours)
      (1) Types and Interpretation of results including predictive values
   e. Strategies for use (30 minutes)

2. How to use handbooks (2 hours)
   a. Information collection
   b. Risk assessment
   c. History, prevalence and management practices
   d. Testing strategy
   e. Management plan development – Link back JD management to existing management and owner goals

3. Uniform Program Standards for the Voluntary Bovine Johne's Disease Program (30 minutes - 1 hour)

4. State specific regulations and program standards (30 minutes)

II. Recertification Training

A. Required Topics

1. Review of Johne's basics

2 Epidemiology update

3. Testing and interpretation
   a. New and emerging tests
   b. Best tests for different scenarios

4. National and State program review, highlighting any changes

5. JD Economics and marketing tips

B. Additional Suggested Special Challenges and Topics

1. Correcting common misconceptions (identified by DJC) – case scenarios

2. Update on research regarding the zoonotic issue

3. Vaccine usage

4. Potential use of Monensin or other Johne's disease "control" products.