

National Veterinary Services Laboratories
Johne's Fecal Pooling Kit
Proficiency Test Summary

1. Composition of proficiency test panel: The panel consists of 25 samples (approximately 6-8 grams) of individually aliquoted fecal samples. The samples are labeled with numbers 1-25. Depending upon the testing key for the pooling kits that testing year, kits will contain a prescribed number of positive and negative samples. This allows for each lab to be tested on five final fecal pools.

2. Cost of proficiency test: [Click here to view current NVSL Catalog of Reagents](#)

3. Storage conditions: Kits are stored at -70°C or lower before shipping and should be stored at -70°C or lower after receipt if not used immediately.

4. Sample preparation/selection criteria: Animals are monitored throughout the year with two to four fecal collections each month to monitor animal shedding status. At the time of proficiency sample preparation, two or three liters of fecal sample is combined and homogenized in either a blender or stomacher. Fecal collections are selected to correspond to high, moderate, and low shedding concentrations of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), or negative with no shedding and/or isolation of MAP. After homogenization, samples are aliquoted into 6-8 gram volumes. After aliquoting, a sample is processed for isolation of MAP, and colony counts are recorded for that particular fecal pool. If a sample falls outside the proposed range, samples are discarded and new satisfactory samples replace the discarded pool. Kits are then assembled and frozen at -70°C. After kits are frozen, two or three kits from each lot are selected and processed for the isolation of MAP and to record colony counts.

5. Panel quality control: Samples are processed for viability count determination prior to and after freezing. Kits are held at -70°C for short-term time frames prior to shipping, and stability testing of the panel has determined that normal shipping and handling conditions do not affect the sample quality. In addition, inter-laboratory evaluation of sample results is considered in the final validation of individual fecal samples.

6. Timing of the proficiency test distribution and data collection: The Johne's Fecal Pooling proficiency test is administered once a year, typically in April or May. A retest procedure is in place that will allow for retesting for failing laboratories, which normally takes place in September or October.

7. Test method: Individual laboratories are free to perform proficiency testing by their method of choice. However, laboratories are required to indicate to the NVSL which testing method they will be conducting (Solid Media, Bactec 460, Bactec 960, TREK ESP, Direct PCR). Individual kits must be utilized for each selected test method should a laboratory request proficiency testing on multiple methods. Laboratories are instructed as to which individual samples are to be pooled for each final pool sample evaluation.

8. Submitting test results: Participants are required to have data submitted for scoring based upon a deadline set according to their detection method. The guidelines state the following: Direct PCR – 4 week submission, Liquid Culture (Bactec 460, Bactec 960, TREK ESP) – 10 week submission, Solid Media – 18 weeks. Results submission forms are supplied to laboratories electronically at the time kits are shipped. Results are submitted either by fax, e-mail, or standard mail. Results are entered into a tracking spreadsheet and submission forms are maintained in a file or binder until the testing period is completed. Laboratories are asked to indicate all or parts of the following based upon their methodology and usual reporting structure: Colony counts (per tube), days to positive (for liquid culture systems), acid-fast staining results, confirmatory PCR results, shedding status classification, and final sample determination as to being positive or negative.

9. Scoring of individual panel pool samples: Individual pools are first scored for indication of being identified as positive or negative. Colony counts (solid media) or days to positive (liquid cultures) indication is also evaluated, but not factored into the final scoring scheme. Results for acid-fast staining and PCR confirmation testing are also examined, but not part of the final scoring.

10. Laboratory pass/fail criteria: A passing score consists of correct identification of three critical samples: 1) high shedder pool, 2) moderate shedder pool, and 3) negative pool, and the correct identification of one of the two remaining pools, for a final minimum score of 80%.

11. Reporting laboratory test scores: Results for each laboratory are reported only to the individual laboratory director. The director is asked to share the results with each individual participant. The Final Report on the Proficiency Panel Test is compiled and sent to laboratory directors within 60-90 days of the final submission deadline.

12. Remedial actions required for failing laboratories: At this time, no retest for pooling is allowed. Laboratories that do not pass are not allowed to participate in program herd testing/certification utilizing a fecal pooling procedure.

13. Special requirements: Restrictions - Laboratories are required to submit a Johne's Disease Pooling Fecal Check Test Order Form to participate in testing each year. Invitation letters and order forms are sent to previously participating laboratories in November or December of the preceding testing year.