National Veterinary Services Laboratories
Johne's Fecal
Proficiency Test Summary

1. Composition of proficiency test panel: The panel consists of 25, approximately 6-8 gram samples, of individually aliquoted fecal samples. The panel contains duplicate or triplicate positive and negative samples within each kit. One positive control sample is also included with each kit. The samples are labeled with numbers 1-26.

2. Cost of proficiency test: Click here to view current NVSL Catalog of Reagents. Additional information on diagnostic reagents, including ordering, payments, shipping details and fees, is located at www.aphis.usda.gov/nvsl. Then click on the Reagents and Proficiency Tests link.

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 demonstrated 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 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 labs, which normally takes place during September or October.

7. Test method: Individual laboratories are free to perform proficiency testing by their method of choice. However, labs 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 lab request proficiency testing on multiple methods.

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, email, 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. Labs 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), acidfast staining results, confirmatory PCR results, shedding status classification, and final sample determination as to being positive or negative.

9. Scoring of individual panel samples: Individual samples are first scored for indication of being identified as positive or negative. For labs utilizing a liquid culture system or direct fecal PCR, the individual samples are only scored as positive or negative. For labs utilizing solid media (Herrold's Egg Yolk), samples are first evaluated as being reported as positive or negative. Individual samples are then evaluated for reported colony counts. For samples which were keyed as "high shedders" or "TNTC", the results are evaluated to check if the reporting lab has identified at least two tubes of media as having counts of TNTC or over 50 colonies on the individual tubes. A final colony count average is also calculated for each sample. Results for acidfast staining and PCR confirmation testing are also examined.

10. Laboratory pass/fail criteria: A passing score consists of (1) identification of 100% of the negatives correctly, (2) identification of 100% of the TNTC (too numerous to count) positives correctly and (3) identification of 70% of the remaining positives correctly to give a minimum score of 85%. For a sample to be considered valid, there must be a 70% consensus among the participating laboratories. For a sample to be considered TNTC, there must be a 50% consensus among the participating laboratories using Herrold's Egg Yolk media.

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: Laboratories that do not pass on the first attempt are given the opportunity for a retest. Failure to pass on the retest means that laboratory is not allowed to participate in program herd testing/certification utilizing the failed method. If a laboratory passed utilizing an additional methodology, the laboratory would be approved for that particular method. Laboratories that fail to detect 50% of the "consensus classified fecal check test TNTC samples" are notified of their proficiency results with the intent to improve their detection techniques during the coming year (Probational Approval). If a laboratory fails to detect these consensus

classified TNTC for the second year they would be required to take additional training at the NVSL.

13. Special requirements: Restrictions: Laboratories are required to submit a Johne's Disease 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.