

National Veterinary Services Laboratories
Johne's Disease (*Mycobacterium paratuberculosis*) Serology
Proficiency Test Summary

- 1. Composition of proficiency test panel:** The panel consists of 25 samples of bovine serum. Individual samples consist of 0.20 ml of serum provided in bottles with rubber stoppers and aluminum seals. Vials are labeled with the sample number, serial number, volume, storage instructions, and the NVSL address.
- 2. Cost of proficiency test:** \$361.00 plus shipping (\$10-US, \$50 Canada, \$150 other).
- 3. Storage conditions:** Short term (up to 7 days) store at $4^{\circ} \pm 2^{\circ}$ C. Long term (over 7 days) store at $<-20^{\circ}$ C in a non-frost free freezer.
- 4. Sample preparation/selection criteria:** The serum panel consists of at least five known strong-positive samples and at least five (5) known negative samples ("critical samples"). The remaining samples ("quantitative samples") are selected to create a panel with an expected range from negative to positive, with emphasis placed on samples near the midrange. Samples may not be used more than twice within the panel. Sample panels consist of well characterized control sera that have been evaluated extensively in the commercial ELISA kits available in the United States for Johne's disease. The panel is retested by the Serology Section of the Diagnostic Bacteriology Laboratory (DBL) prior to shipment to participating laboratories to ensure that the samples are giving expected results.
- 5. Panel quality control:** Samples are monitored for sterility, stability, and reproducibility. Bulk volumes of serum are filtered using a 0.22 μ m filter and tested for sterility prior to bottling.
- 6. Timing of the proficiency test distribution and data collection:** The Johne's Disease Serology Proficiency Test is administered once a year, generally in June.
- 7. Test method:** Performance and interpretation of the Johne's Disease Serology Proficiency Test should be conducted using the manufacturer's kit inserts for the commercial ELISAs currently available in the United States (PARACHEK Johne's Absorbed EIA kit, Prionics USA, Inc.; and HerdChek *Mycobacterium paratuberculosis* Antibody Test Kit, IDEXX Laboratories). Laboratories may choose to perform methods other than ELISA, such as their own in-house assay, agar gel immunodiffusion (AGID), or by complement fixation test (CFT) for internal quality assurance. However, only ELISA results will be used to approve the laboratory.
- 8. Submitting test results:** Participants are required to submit data for scoring no more than four (4) weeks after panel distribution. Results are reported to the Johne's Disease Proficiency Test Coordinator by fax, e-mail, or mail. Reporting results via e-mail using electronic score sheets provided is preferred in order to increase efficiency and decrease

transcription errors. The electronic form calculates the means of the serum controls and provides qualitative (positive/negative) and quantitative (OD values and/or S/P values depending on the specific assay) results for each sample with automatic calculations using formulas in the shaded columns.

9. Scoring of individual panel samples: For each sample, the ELISA result is scored based on a combination of correctly identifying known positive and negative samples (critical samples), and a statistical analysis for samples with result values that lie close to the positive threshold (quantitative samples). Critical samples must be identified correctly as positive or negative. Quantitative samples are considered to be passing if the results fall within an acceptable range, as determined by statistical analysis provided by the Centers for Epidemiology & Animal Health (CEAH).

10. Laboratory pass/fail criteria: To receive a final passing score, all critical samples must be correctly identified as either positive or negative, and $\geq 90\%$ of the quantitative samples must be within range. Laboratories submitting a passing proficiency test are approved to perform the ELISA for Johne's disease for official purposes. Each individual that performs the test is not required to pass the test. Laboratories that perform the test using other methods than the commercially available ELISAs are graded on a qualitative basis for all samples, and must correctly identify all of the critical samples and $\geq 90\%$ of the remaining samples.

11. Reporting laboratory test scores: Results for each laboratory are reported to the individual laboratory director or Johne's Disease Proficiency Test contact. Final results are compiled and reported within 60-90 days of the deadline for receipt of participants' results. Individual laboratory scores are reported only to that laboratory, and summary results from all laboratories are provided as part of the final report. A list of Approved Laboratories is published on the NVSL and USAHA web sites.

12. Remedial actions required for failing laboratories: Laboratories that do not pass on the first attempt are offered a retest. Failure to pass on the retest does not preclude a laboratory from performing an ELISA for Johne's Disease, but laboratories must successfully pass the proficiency test in order to perform official testing as part of the Voluntary Bovine Johne's Disease Control Program. After a failure on the retest, a recommendation to the laboratory is made to consult the manufacturer for advice or to seek remedial training for the test performer.

13. Special requirements: The Johne's Disease Serology Proficiency Test is intended to provide for certification of the proficiency of laboratories to test cattle by commercial ELISAs available in the U.S. for Johne's Disease. This certification is not required to purchase or perform the Johne's ELISAs. The purpose of the proficiency test is to provide a means for approval of testing laboratories by the NVSL to perform the Johne's ELISA for official testing as part of the Johne's Disease Control Program.