

National Veterinary Services Laboratories
Johne's Disease (*Mycobacterium avium* subspecies *paratuberculosis*) Serology - Milk ELISA
Proficiency Test Summary

1. **Composition of proficiency test panel:** The panel consists of 20 to 25 samples of milk from cattle. Individual samples consist of 1.0 ml of milk provided in cryogenic vials. Vials are labeled with the sample number, serial number, storage instructions, and the NVSL address.
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:** Short term storage (up to 7 days) store at $4^{\circ} \pm 2^{\circ}$ C. Long term (over 7 days) store at $<-20^{\circ}$ C in a freezer.
4. **Sample preparation/selection criteria:** The milk panel consists of four (4) known strong-positive samples and at least four (4) known negative samples ("critical samples"). The remaining 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 milk that has been evaluated in the commercial ELISA kits for milk available in the United States for Johne's disease. The panel is retested by the Brucella Mycobacteria Reagents Team (BMRT) of the Diagnostic Bacteriology Laboratory (DBL) prior to shipment to participating laboratories to ensure the samples are giving expected results.
5. **Panel quality control:** Samples are monitored for stability and reproducibility. Milk samples have been treated with a bacteriostatic antibiotic to prevent deterioration of the sample. Samples are not checked for sterility due to the unstable nature of the milk sample.
6. **Timing of the proficiency test distribution and data collection:** The Johne's Disease Milk ELISA Proficiency Test is administered once a year, generally in March.
7. **Test method:** Performance and interpretation of the Johne's Disease Milk ELISA Proficiency Test should be conducted using the manufacturer's kit inserts for the commercial ELISAs currently available in the United States (*Mycobacterium paratuberculosis* Antibody Test Kit - PARACHEK[®] 2, Prionics USA, Inc.; and *Mycobacterium avium* subsp. *paratuberculosis* Antibody Test Kit, IDEXX Laboratories). Laboratories may choose to perform methods other than these ELISAs, such as their own in-house assay for internal quality assurance.
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 or e-mail.
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). These must be correctly identified as positive and negative. The remaining sample results are determined by $\geq 80\%$ consensus between laboratory results of positive, negative, or suspect.

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 remaining samples must be correctly identified. Laboratories submitting a passing proficiency test are approved to perform the milk ELISA for Johne's disease for official purposes. Each individual who 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 in the same manner.
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 30-60 days of the deadline for receipt of participants' results. Individual laboratory scores are reported only to that laboratory, and summary results of 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 a milk 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 Milk ELISA Proficiency Test is intended to provide for certification of the proficiency of laboratories to test cattle by commercial milk ELISAs available in the U. S. for Johne's disease. This certification is not required to purchase or perform the Johne's milk 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 milk ELISA for official testing.