National Veterinary Services Laboratories
Avian Influenza (AI) Real-Time RT-PCR
Proficiency Test Summary

1. Composition of proficiency test panel: Each panel consists of ten 1.25-ml samples of beta-propriolactone (BPL) inactivated Avian Influenza (Low Pathogenic AI subtypes H1, H5 and H7). The panel contains blind duplicates, serial dilutions, and negative extraction controls (Tris-Buffered Tryptose Broth (TBTB)). The samples are coded with numbers 1 through 10.

2. Cost of proficiency test: $361.00 plus shipping ($10-US, $50 Canada, $150 other). No cost to approved NAHLN laboratories.

3. Storage conditions: -20 C or lower.

4. Sample preparation/selection criteria: A limit of detection assay is performed on each panel member. Samples with high, medium, and low concentrations of the target analyte are chosen for incorporation into the panel.

5. Panel quality control: Limit of detection is conducted to determine high, medium, and low analyte specimens. Following selection of the specimens, testing is conducted to determine the expected cycle threshold (Ct) for each specimen with real-time instrumentation that is described within NVSL SOP AVPRO1510.03.

6. Timing of the proficiency test distribution and data collection: The AI rRT-PCR proficiency tests are administered annually in January.

7. Test method: Performance and interpretation of the avian influenza proficiency test should be conducted using the real time RT-PCR assay as outlined in NVSL SOP AVPRO1510.03. All samples are screened using the AI Matrix primer and probe set, and Matrix positives are tested by the H5 and H7 subtype assays.

8. Submitting test results: Participants are required to have data submitted for scoring no more than four (4) weeks after panel distribution. Results are reported to the administering laboratory, the Diagnostic Virology Laboratory (DVL), at the NVSL, by fax. Results for all laboratories are kept at the testing laboratory office.

9. Scoring of individual panel samples: For each sample, a participant is considered as passing if the unknown samples are identified correctly (e.g., identification of negatives, Matrix positives, H5 positives, and H7 positives).

10. Laboratory pass/fail criteria: The final score is based on the identification of positive and negative samples. Results are compiled and sent to the NVSL statistician for statistical analysis. Passing scores are based on a 95% confidence interval for the group. The number of misses allowed each year varies based on submitted results. Misses are

not cumulative for each assay (Matrix, H5, H7). Historically, no more than two (2) misses on one assay have been allowed to achieve a passing score. Successful completion of the Matrix, H5 and H7 assays are necessary for approval to conduct testing with the AI rRT-PCR assay.

11. Reporting laboratory test scores: Results for each laboratory are reported only to the respective 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 along with a letter of approval or failure within 60-90 days of the receipt of participants' results. Approval letters are mailed separate from failure letters.

12. Remedial actions required for failing laboratories: Individual participants from a laboratory must pass all three (3) parts of the proficiency test in order for that person to perform any AI rRT-PCR assay. Individual personnel from a laboratory that do not successfully complete the proficiency test on the first attempt are given a retest. Failure to successfully complete the proficiency test on the retest means that participant is not allowed to conduct testing by the AI rRT-PCR assay. If all personnel from a laboratory fail, the laboratory is not approved for testing for that disease. Those laboratories that show repeated fail attempts are encouraged to contact the administering laboratory for discussion of potential areas of concern. These laboratories are asked to test again in the next round of testing. If requested by the laboratory, additional training samples may be provided to the laboratory for practice purposes.

13. Special requirements: Only laboratory personnel, who have successfully completed an approved Avian Influenza/Newcastle's Disease Virus rRT-PCR training course, are eligible to participate in this proficiency test.