National Veterinary Services Laboratories
Avian Paramyxovirus-1 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 Paramyxovirus-1 (LaSota and Texas G.B.). 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).

3. Storage conditions: The panel is to be stored at -20°C or colder until use.

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 testing 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 AVPRO1505.

6. Timing of the proficiency test distribution and data collection: The APMV-1 rRT-PCR proficiency tests are administered annually in January.

7. Test method: Performance and interpretation of the avian paramyxovirus-1 proficiency test should be conducted using the real time RT-PCR assay as outlined in NVSL SOP AVPRO1505. All samples are screened using the APMV-1 Matrix primer and probe set, and Matrix positives are tested by the vNDV pathotyping assay.

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 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 (identification of negatives, Matrix positives, and v-NDV 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 submitted results. Misses are not cumulative for each assay (Matrix, vNDV). Historically, no more than two (2) misses on

one assay have been allowed to achieve a passing score. Successful completion of both the Matrix and vNDV assays is necessary for approval to conduct testing with the APMV-1 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 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 results in that participant not being allowed to conduct testing by the APMV-1 rRT-PCR assay. If all personnel form a laboratory fail, the laboratory is not approved for testing for that disease. Those laboratories that show repeated failed 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 those laboratory personnel who have been trained at NVSL, or an alternatively identified NAHLN laboratory (as approved by NAHLN), and successfully proficiency tested are eligible for performing proficiency panel testing.