

**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-propiolactone (BPL) inactivated Avian Paramyxovirus-1 (LaSota and Texas G.B.) and avian influenza viruses. 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:

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.

No cost to approved NAHLN laboratories.

3. Storage conditions: -20 C or lower in a non-frost free freezer.

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 the current version of SOP-AV-1520 (Smart Cycler), SOP-AV-1521 (AB 7500 Fast), SOP-AV-1523 (AB 7900 HT), and SOP-AV-1524 (Roche LightCycler 480).

6. Timing of the proficiency test distribution and data collection: The APMV-1 rRT-PCR proficiency tests are administered annually in January. Results are collected 4 weeks after distribution.

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 the current version of SOP-AV-1505 and the associated instrument-specific documents. 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 or e-mail. 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 vNDV 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. The final score is based on the identification of positive and negative samples. Results are compiled and analyzed by statistical methods. The minimum passing score is 90%. Successful completion of both the Matrix and vNDV assays is necessary for approval to conduct testing with the APMV-1 rRT-PCR assay. Successful completion implies the individual is proficient on all test methods outlined and described in the current version of SOP-AV-1505 and the associated documents.

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 non-approval letters. A copy of the results for all National Animal Health Laboratory Network (NAHLN) laboratories is sent to the NAHLN Program Office.

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 from 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. Additional training may be provided if requested by the NAHLN laboratory, by the NVSL and/or by the NAHLN Program Office.

13. Special requirements: Only laboratory personnel who have successfully completed an approved Avian Influenza/Newcastle Disease Virus rRT-PCR training course are eligible to participate in this proficiency test. Training may be administered by the NVSL or through the Train-the-Trainer program. Training received at a laboratory other than the NVSL must be approved by the NAHLN Program Office in conjunction with NVSL. Participating NAHLN laboratories must comply with provisions outlined in the NAHLN Laboratory Qualification Checklist for Membership of a Veterinary Diagnostic Laboratory.