National Veterinary Services Laboratories
Foot-and-Mouth Disease Virus
Proficiency Test Summary

1. Composition of proficiency test panel: The panel consists of twelve 500-µl samples of an armored RNA (aRNA) construct. The panel contains blind duplicate samples. Included with the panels are positive (aRNA in Dulbecco's Modified Eagle Medium (DMEM)) and negative extraction controls (DMEM), as well as a positive amplification control (extracted RNA obtained from aRNA contstruct). The samples are assigned a position within the panel using a random number generator. Multiple arrangements are prepared for each lot of panels. Each sample within a panel arrangement is labeled with a number from 1-12.

2. Cost of proficiency test to users: No charge to approved laboratories.

3. Storage conditions: Upon arrival at your laboratory, the proficiency panels should be stored at -70°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. Each panel member is tested 20 times by a minimum of three technicians.

5. Panel quality control: Stability of the panels is monitored throughout the lifespan of the panels**.** Prior stability testing has determined that normal shipping and handling conditions do not alter the performance of the components included in each panel. Ten percent of each lot of proficiency panels is used to determine intra- and inter-laboratory variation. For inter-laboratory variation, results from proficiency tests may be used. For each new lot of panels, a set of panels is sent to selected laboratories for preliminary data collection purposes.

6. Timing of the proficiency test distribution and data collection: The FMD proficiency test is administered annually in September.

7. Test method: Performance and Interpretation of the Foot-and-Mouth Disease proficiency test should be conducted using the real time RT-PCR assay as outlined in PVSSOPs 4000, 4001 and 4002 (Cepheid Smart Cycler), PVSSOP4005 (Applied Biosystems ABI7900 or PVSSOP4004 (Applied Biosystems ABI7500).

8. Submitting test results: This proficiency test is administered to individuals rather than laboratories. Each individual participant is required to have data submitted for scoring no more than Four (4) weeks after panel distribution. Results are reported to the test administrative office at the Proficiency and Validation section at the Foreign Animal Disease Diagnostic Laboratory (FADDL) by fax or e-mail. Archived copies of the PCR run are e-mailed or retained by the test participant for three (3) months. Results for all

laboratories are sent to the NAHLN Coordinator office and a copy is kept at the testing laboratory office.

9. Scoring of individual panel samples: For each sample, a participant is considered as passing if the known negative sample is identified as negative. A participant is considered as passing if the known positive sample is identified as either positive or inconclusive.

10. Laboratory pass/fail criteria: The final score is based on the identification of positive and negative samples. The results are determined by identification of positive and negative samples for each disease. Interpretation is weighted as 100% of the grade. The Ct value results are analyzed but are not included in scoring the applicant on the proficiency test. However, if the resultant Ct values are significantly higher for positive samples or lower for negative samples than "expected ranges" set forth for each instrument during testing at the FADDL, then the laboratory directors will be notified and an attempt to troubleshoot the discrepancy will be undertaken.

A final score of ≥ 90% is considered passing.

11. Reporting laboratory test scores: Results for each laboratory are reported only to the individual 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 within 60-90 days of the receipt of participants results.

12. Remedial actions required for failing laboratories: Individual personnel from a laboratory that do not pass on the first attempt are given a retest. Failure to pass on the retest means that participant is not allowed to administer the failed procedure. If all personnel from a laboratory fail, the laboratory is not cleared for testing for that disease. Those laboratories that show repeated fail attempts are encouraged to contact Proficiency and Validation Services section at the FADDL 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 panels may be provided to the laboratory for practice purposes.

13. Special requirements: Only those laboratory personnel who have been trained at FADDL and/or trained as part of the FMD "Train the Trainer" program are eligible for performing proficiency panel testing.