

**United States Department of Agriculture  
Center for Veterinary Biologics  
Testing Protocol**

**SAM 120**

**Supplemental Assay Method for the *In vitro* Potency Assay of Bovine  
Respiratory Viruses in Killed Vaccines**

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Supplemental Assay Method for the *In vitro* Potency Assay of Bovine Respiratory Viruses in Killed Vaccines

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Supplemental Assay Method for the *In vitro* Potency Assay of Bovine Respiratory Viruses in Killed Vaccines

**1. Introduction**

This Supplemental Assay Method (SAM) describes an *in vitro* assay for determining the potency of killed vaccines containing Bovine Rhinotracheitis (BRV), Bovine Virus Diarrhea (BVD), Bovine Parainfluenza-3 (PI<sub>3</sub>), and Bovine Respiratory Syncytial Viruses (BRSV) relative to a reference vaccine.

**2. Materials**

**2.1** 96-well microtiter plate

**2.2 Coating buffer:** 0.05 M sodium carbonate/bicarbonate pH 9.6. Store at 4°C; use within 5 days.

**2.2.1** 1.59 g Na<sub>2</sub>CO<sub>3</sub>

**2.2.2** 2.93 g NaHCO<sub>3</sub>

**2.2.3** Distilled H<sub>2</sub>O, q.s. to 1 L

**2.2.4** Adjust pH to 9.6 ± 0.1.

**2.3** Anti-viral agent capture antibody, using 1 capture antibody only, and assay 1 viral fraction in each test. Reference quantities are available from the National Veterinary Services Laboratories (NVSL). Dilute in coating buffer according to instructions.

**2.3.1** BRV antibody

**2.3.2** BVD antibody

**2.3.3** Bovine PI<sub>3</sub> antibody

**2.3.4** BRSV antibody

**2.4 Blocking buffer:** 1% casein in coating buffer. Store at 4°C; use within 5 days.

**2.4.1** 1 g casein

**2.4.2** 100 mL coating buffer

**2.4.3** Heat 2 to 3 minutes until boiling in a microwave on high setting.

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**2.5 0.01 M PBS.** Store at 4°C.

**2.5.1** 1.33 g Na<sub>2</sub>HPO<sub>4</sub>

**2.5.2** 0.22 g Na<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>•H<sub>2</sub>O

**2.5.3** 8.5 g NaCl

**2.5.4** Distilled H<sub>2</sub>O, q.s. to 1 L

**2.5.5** Autoclave 15 minutes at 121°C.

**2.6 Diluent buffer:** 1% casein in 0.01 M PBS. Prepare fresh each time test is performed.

**2.6.1** 1 g casein

**2.6.2** 100 mL 0.01 M PBS

**2.6.3** Heat 2 to 3 minutes until boiling in a microwave on high setting.

**2.7** Wash reagent: 0.05% Tween-20 in 0.01 M PBS

**2.8 Secondary antibody:** Available from the NVSL unless noted. Dilute in diluent buffer according to instructions.

**2.8.1** BRV monoclonal antibody (MAb):

**1.** 1B8

**2.** 2H6

**2.8.2** BVD biotin-labeled polyclonal antibody

**2.8.3** PI<sub>3</sub> MAb:

**1.** 240-12D

**2.** 260-10B

**2.8.4** BRSV MAb 8G12

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**2.9 Conjugate**

**2.9.1** Horseradish peroxidase-labeled goat anti-mouse IgG, diluted 1:3000 in 0.01 M PBS, is to be used with the BRV, PI<sub>3</sub>, and BRSV MAbs.

**2.9.2** Peroxidase conjugated streptavidin, diluted 1:4000 in 0.01 M PBS, is to be used with biotin-labeled polyclonal BVD antibody.

**2.10** Substrate: 2,2'-azino-di (3-ethylbenzthiazoline sulfonic acid) (ABTS)

Mix equal parts of Solution A and Solution B as supplied just before use in the *in vitro* test.

**2.11** Reference vaccine(s) to be supplied by the licensee. The reference vaccine(s) should have the same dose size and composition as the test serial, be validated in a host immunogenicity study and shown to work in a parallel line assay. Refer to the Guidelines for measuring the relative potency of veterinary biologics by ELISA, dated April 26, 1991.

**3. Performance of the Test**

If the test serial vaccine is treated to release antigen from adjuvant, it shall be a method acceptable to the Animal and Plant Health Inspection Service (APHIS) and shall be applied to the reference vaccine as well.

**3.1** Coat microtiter plate with 100 µL/well of capture antibody diluted in coating buffer. Cover with sealing tape. Incubated overnight at 4°C.

**3.2** Decant capture antibody. Blot inverted plate on paper towels. Add 100 µL/well of blocking buffer. Incubate 1 hour at room temperature.

**3.3** Rinse microtiter plate 3 times using 200 µL/well of wash reagent. Remove residual wash reagent by blotting inverted plate on paper towels after 3rd wash. Prevent drying of plate between reagent additions.

**3.4** Prepare serial twofold dilutions of the reference and test serial vaccines in a separate dilution plate.

**3.4.1** Add 150 µL/well of diluent buffer to rows B-H of a 96-well plate.

**3.4.2** Add 300 µL/well of reference vaccine to A1 through A3 of the dilution plate. Add 300 µL/well of the test serial vaccine to A4 through A6 of the dilution

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plate. Additional test serials may be tested in A7 through A9 and A10 through A12.

**3.4.3** Using a multichannel pipette, transfer 150  $\mu\text{L}$  of row A to row B. Thoroughly mix contents of wells in row B. Transfer 150  $\mu\text{L}$  to the corresponding wells in row C.

**Note: One set of pipette tips may be used for diluting the reference and serial vaccines.**

**3.4.4** Thoroughly mix contents of wells in row C. Transfer 150  $\mu\text{L}$  to the corresponding wells in row D.

**3.4.5** Continue serial twofold dilutions through row G. After thoroughly mixing the contents in wells G, discard 150  $\mu\text{L}$  from row G. The dilutions of the vaccines range from undiluted to 1:64.

**Note: Other dilution schemes for the reference vaccine and test serial may be used to obtain optimal optical density (OD) readings.**

**3.4.6** Row H is the blanking row.

**3.5** Use the multichannel pipette to transfer 100  $\mu\text{L}$  aliquots from row H of the dilution plate to the corresponding row H of the capture antibody coated plate. Continue transfer of all rows of the dilution plate from row G through row A. One set of pipette tips may be used to transfer. Incubate 1 hour at 37°C on a microtiter plate shaker.

**3.6** Rinse plate again as in **Step 3.3**.

**3.7** Add 100  $\mu\text{L}$ /well of secondary antibody diluted in diluent buffer to all wells of the plate. Incubate 1 hour at 37°C.

**3.8** Rinse plate again as in **Step 3.3**.

**3.9** Add 100  $\mu\text{L}$ /well of conjugate diluted in 0.01 M PBS. Incubate 30 minutes at 37°C.

**3.10** Rinse plate 3 times with 0.01 M PBS without Tween-20. Remove residual PBS by blotting inverted plate on paper towels after the 3rd wash.

**3.11** Add 100  $\mu\text{L}$ /well of ABTS. Incubate 45 minutes at room temperature.

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**3.12** Read plate on an ELISA reader with 405 nm for the test filter and 490 nm for the reference filter.

**3.13** Blank reader on well(s) containing all reagents except the fraction being tested (row H). If reader blanking is not done, the mean OD value obtained from the blank(s) should be subtracted from all other OD values before any data analysis.

**4. Interpretation of the Test Results**

A test that results in no valid lines is considered a no test and may be repeated. The reported relative potency (RP) of the test serial is the highest RP from the top 3 scores calculated using SoftMax Pro 6.3 GxP. Test serials with  $RP \geq 1.0$  are considered satisfactory. Retest procedures are covered under title 9, *Code of Federal Regulations* (9 CFR), part 113.8.

Test serials with a  $RP < 1.0$  may be retested up to a total of 3 times. At least 50% of all valid tests must be satisfactory for the test serial to be considered satisfactory. If the test serial is not retested, the serial is considered unsatisfactory.

**5. Report of Test Results**

Record all test results on the test record.

**6. References**

Title 9, *Code of Federal Regulations*, part 113.8, U.S. Printing Office, Washington, DC.

**7. Summary of Revisions**

**Version .04**

- **The coversheet and contact information have been updated.**

**Version .03**

- The Contact information has been updated; however, the Virology Section has elected to keep the same next review date for the document.

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**Version .02**

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the following changes were made to the document:

- The Contact information has been updated.

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