United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 408

Supplemental Assay Method for Titrating Tissue Culture Adapted Vaccine Strains of Infectious Bursal Disease Virus

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1. Introduction

This Supplemental Assay Method (SAM) describes the titration of vaccine strains of tissue culture adapted infectious bursal disease virus (IBDV) in primary chick embryo fibroblast cell cultures (1°CEF).

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

2.1.1 Water-jacketed incubator with a humidified 5 ± 1% CO_2 atmosphere and temperature set at 37º± 2°C (Forma Scientific, Model No. 3158)

2.1.2 Vortex mixer (Thermolyne Maxi Mix II, Model No. M37615)

2.1.3 Microliter pipette (Rainin Pipetman, P1000)

2.1.4 Laminar Flow Biological Safety Cabinet (NuAire Inc., Labgard)

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below. All reagents and supplies must be sterile.

2.2.1 24-well tissue culture treated plates, planted with 1 mL of 1°CEFs suspension per well at a concentration of approximately 400,000 cells/mL. The growth medium is M-199/F10 with 2-3% FBS, 1% L-glutamine, and 2 µg/mL Fungizone. 1°CEFs are from susceptible specific pathogen free (SPF) embryonating chicken eggs.

2.2.2 M-199/F-10 media (National Centers for Animal Health (NCAH) Media #20012):

M199/F10 Formula

Medium 199	47.74 g
1x F-10 Nutrient Mixture	5000 mL
Bacto Tryptose Phosphate Broth	14.8 g
Penicillin G	0.613 g
Streptomycin	1.0 g

Sodium Bicarbonate	22 g
HEPES	59.85 g
Sterile, distilled, or deionized water	10.5 L

All ingredients are mixed, pH is adjusted to 7.2, and the solution is filter sterilized. The pH is checked again after sterilization. The solution is then dispensed into appropriate containers.

2.2.3 Sterile distilled or deionized water, 100 mL aliquots in 100-mL serum vials with rubber stoppers and aluminum caps

2.2.4 Needle, 1 1/2-inch x 18-gauge

2.2.5 LuerLok disposable syringe, 5- or 10-cc

2.2.6 Sterile pipette tips, Rainin P100-P1000

2.2.7 Sterile glass test tubes, 16 x 125-mm

2.2.8 Fetal Bovine Serum (FBS)

2.2.9 L-Glutamine (200 mM)

2.2.10 Pipettes, various volumes

2.2.11 Fungizone

3. Preparation for the Test

3.1 Personnel qualifications/training

Personnel must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling and disposal of biological agents, reagents, tissue culture samples, and chemicals. Personnel must also have knowledge of safe operating procedures and policies; and training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

Operate all equipment/instrumentation according to manufacturers' instructions and monitor in compliance with current corresponding standard operating policies/procedures (SOPs) or equivalent.

3.3 Preparation of reagents/control procedures

Prepare reference viruses in the same manner as sample preparation (see **Section 3.4**).

3.4 Preparation of the sample

Preparation of vaccine for titration: rehydrate or dilute vaccine in 100 mL of sterile deionized water. Mix thoroughly. If necessary, further dilute the vaccine so that 1 dose is contained in a volume of 0.1 mL.

4. Performance of the Test

To prepare the dilution and inoculate the plates, use maintenance media consisting of M199/F10 media with 1% FBS, 1% L-Glutamine, and 2 µg/mL of Fungizone to make dilutions for both the vaccine and positive control virus. Make tenfold serial dilutions of the vaccine and the positive control virus encompassing the range of the expected titer. Use at least 4 dilutions for a titration. Inoculate 5 wells with 0.1 mL per well for each dilution. The sixth well of each row remains uninoculated and serves as a cell control. Incubate in a humidified atmosphere of approximately 5 ± 1% CO_2 at 37º± 2°C.

5. Interpretation of the Test Results

5.1 Controls

The titer of the positive control must be within the established range for the test results to be valid. Uninoculated negative control cells are maintained to monitor the integrity of the cell culture system.

5.2 Calculating the titer

Microscopically examine the plates daily and track the development of typical IBDV cytopathology including refractile cells. The negative control wells must remain normal throughout the test. On day 7 postinoculation, calculate the 50% endpoint of infectivity using the Reed-Muench method. This value will represent the titer per dose.

5.3 Retests

Conduct retests as required by title 9, *Code of Federal Regulations* (9 CFR), section 113.8(b), and also by the requirements in the firm's current Outline of Production, Part V.

5.4 Evaluation of test results

5.4.1 The 9 CFR 113.8(b) defines the criteria for a satisfactory/unsatisfactory serial.

5.4.2 The firm's requirements of minimum release/stability titers for each IBD vaccine are listed in the current Outline of Production, Part V, for the specific product code.

6. Report of Test Results

Titers are reported out as Tissue Culture Infective Dose 50% Endpoint ($TCID_{50}$) per bird dose. Report the results as satisfactory or unsatisfactory.

7. Summary of Revisions

Version .06

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

Version .05

- The Contact information has been updated.

Version .04

- The Contact information has been updated.

Version .03

- The Contact has been changed from Scott Taylor and Karen Wineland to Sheridan Booher and Danielle Koski.
- **2.2.1:** Information has been included that pertains to the concentration of FBS, L-Glutamine, and Fungizone that should be added to the growth media.
- **2.2.2:** The M199/F10 formula has been added.
- **2.2.8:** This section has been removed because it was a duplicate listing of equipment in **Section 2.2.1**.

- **3.3:** The phrase "see **Section 3.4**" has been added.
- **3.4:** "Or diluted" has been added to encompass those vaccines not lyophilized.
- **4.1:** Clarification has been added on what the maintenance media consists of.
- **5.3:** The phrase "also by" has been added and the phrase "of minimum release" has been removed.

Version .02

This document was rewritten to clarify 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 introduction has been revised for clarity.
- **2.1** Many items listed were unnecessary to list and have been removed from the document for clarity.
- **2.2** Changes in the reagents/supplies used have been made to the items previously listed for clarity.
- **2.2** The Dulbecco's PBS and trypsin solution (0.25%) have been removed. The growth medium has been removed and replaced with a maintenance medium.
- **2.2.16** The section on cell cultures has been deleted from the document.
- **4.1** The use of the maintenance medium for the vaccine and positive reference virus has been added for clarity.
- **5.2** This section has been revised for clarity.
- **6.** This section has been revised to provide clarification of reporting test results.