



SEPTEMBER 6, 2006

United States  
Department of  
Agriculture

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Washington, DC  
20250

## VETERINARY SERVICES MEMORANDUM NO. 800.93

Subject: Supplemental Assay Methods

To: Veterinary Services Management Team, Biologics Licensees, Permittees,  
and Applicants, Directors, Center for Veterinary Biologics

### I. PURPOSE

The purpose of this memorandum is to present a current list of Supplemental Assay Methods (SAMs) which describe procedures, methods, and techniques used by the Center for Veterinary Biologics (CVB) for official assays of biological products according to 9 CFR [113.2\(a\)](#).

### II. CANCELLATION

This memorandum cancels Veterinary Biologics Memorandum No. 800.93 dated February 2, 2006, and the Listing of Supplemental Assay Methods dated January, 2006.

### III. APPLICATION

- A. SAMs contain detailed instructions for conducting officially required tests. They do not supersede the requirements in the [9 CFR](#) or those in filed Outlines of Production specifically requiring alternative procedures for the licensee or CVB.
- B. CVB must follow SAMs. Revised SAMs must be issued before implementation by CVB.

### IV. IDENTIFICATION OF SAMs

- A. CVB will utilize a standardized title page format on all SAMs which includes the testing protocol heading, the descriptive title of the document, the date of the most recent version, the number, the standard requirement to which it applies, and the name of a person who has agreed to act as primary contact for comments and questions about the document.
- B. Reference to these Supplemental Assay Methods should be made by the letters "SAM" and the applicable number.
- C. CVB will number revisions of SAMs the same as the original and show the new date of issuance with the date of the one being superseded.



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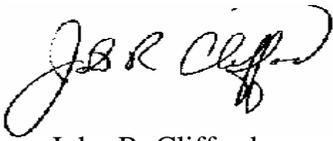
Federal Relay Service  
(Voice/TTY/ASCII/Spanish)  
1-800-877-8339

## **V. PREPARATION AND DISTRIBUTION**

CVB will prepare and distribute SAMs. Initial distribution will be made electronically or by hardcopy to licensees, permittees, applicants, and appropriate Veterinary Services personnel. You may obtain additional copies by accessing the CVB home page at <http://www.aphis.usda.gov/vs/cvb>, or by sending a request to the CVB, 1800 Dayton Avenue, P.O. Box 844, Ames, Iowa 50010.

## **VI. AVAILABILITY**

CVB will periodically issue notices of the addition, revision, or deletion of SAMs. CVB will maintain a current listing of SAMs on the CVB home page.

A handwritten signature in black ink, appearing to read "John R. Clifford". The signature is written in a cursive style with a large initial "J" and a long, sweeping underline.

John R. Clifford  
Deputy Administrator  
Veterinary Services

Enclosure

LISTING OF SUPPLEMENTAL ASSAY METHODS

August 2006

100 Series

- 101 Supplemental Assay Method for Titration of Bovine Viral Diarrhea Virus in Vaccines  
June 6, 2005
- 102 Supplemental Assay Method for Titration of Parainfluenza-3 Virus in Vaccines  
January 1982
- 104 Supplemental Assay Method for Isolation of Parainfluenza-3 Virus from Nasal Secretions  
July 29, 1983
- 105 Supplemental Assay Method for Titration of Infectious Bovine Rhinotracheitis Virus in Vaccines  
April 4, 2005
- 107 Supplemental Assay Method for Titration of Neutralizing Antibody (Constant Serum - Varying Virus Method)  
June 14, 1971
- 108 Supplemental Assay Method for the Detection of Extraneous Bovine Viral Diarrhea Virus in Modified-Live Vaccines  
July 17, 2006
- 109 Supplemental Assay Method for Titration of Bovine Rhinotracheitis Neutralizing Antibody (Constant Virus-Varying Serum Method)  
February 8, 2006
- 110 Supplemental Assay Method for Titration of Eastern, Western, and Venezuelan Equine Encephalomyelitis Antibody Virus Neutralizing Antibodies  
December 20, 2004

- 112            Supplemental Assay Method for Titration of Bovine Virus Diarrhea  
Neutralizing Antibody (Constant Virus - Varying Serum Method)  
1984
- 113            Supplemental Assay Method for Titration of Parainfluenza-3 Neutralizing  
Antibody (Constant Virus - Varying Serum Method)  
August 19, 1983
- 114            Supplemental Assay Method for Titration of Porcine Transmissible  
Gastroenteritis Virus  
June 6, 2005
- 115            Supplemental Assay Method for Titration of Bovine Virus Diarrhea  
Neutralizing Antibody (Constant Serum - Varying Virus Method)  
June 1985
- 116            Supplemental Assay Method for Phenotypic Examination of Pseudorabies Virus  
for Thymidine Kinase Activity by a Plaque Selection Method  
April 4, 2005
- 117            Supplemental Assay Method for Titration of Pseudorabies Virus Neutralizing  
Antibody (Constant Virus -Varying Serum Method)  
June 6, 2005
- 118            Supplemental Assay Method for Titration of Pseudorabies Virus in Vaccines  
April 4, 2005
- 120            Supplemental Assay Method for the *In Vitro* Potency Assay of Bovine  
Respiratory Viruses in Killed Vaccines  
November 1, 1991
- 121            Supplemental Assay Method for Titration of Porcine Rotavirus in  
Modified-Live Vaccines  
June 6, 2005



- 203 Supplemental Assay Method for Potency Testing *Clostridium perfringens*  
Type D Epsilon Antigen  
September 12, 2005
- 204 Supplemental Assay Method for Potency Testing *Clostridium perfringens*  
Type D Antitoxins  
June 20, 2005
- 207 Supplemental Assay Method for Potency Testing *Clostridium novyi* Type B  
Alpha Antigen  
September 8, 2005
- 209 Supplemental Assay Method for Potency Testing Products Containing  
*Clostridium haemolyticum* Antigen  
June 20, 2005
- 212 Supplemental Assay Method for Potency Testing *Clostridium sordellii* Antigen  
September 8, 2005
- 213 Supplemental Assay Method for Potency Testing of *Clostridium botulinum*  
Type C Bacterin-Toxoids  
September 23, 1980
- 214 Supplemental Assay Method for Immunogenicity and Spore Count Testing of  
Anthrax Spore Vaccine-Nonencapsulated  
September 8, 2005
- 216 Supplemental Assay Method for Potency Testing the Potency of Commercial  
Equine Tetanus Antitoxin  
August 14, 1992
- 217 Supplemental Assay Method for Potency Testing of Tetanus Toxoid by ELISA  
June 7, 2006

300 Series

- 302 Supplemental Assay Method for Titration of Canine Distemper Virus in Primary Cell Culture  
June 6, 2005
- 303 Supplemental Assay Method for the Titration of Distemper Virus in Embryonated Chicken Eggs  
June 6, 2005
- 304 Supplemental Assay Method for Titration of Canine Hepatitis Virus in Primary Canine Kidney Cell Culture  
June 6, 2005
- 305 Supplemental Assay Method for the Titration of Feline Panleukopenia Virus in Cell Culture  
June 6, 2005
- 306 Supplemental Assay Method for the Titration of Feline Calicivirus in Cell Culture  
April 4, 2005
- 307 Supplemental Assay Method for the Titration of Feline Rhinotracheitis Virus in Cell Culture  
April 4, 2005
- 308 Supplemental Assay Method for Potency Testing of Inactivated Rabies Vaccine in Mice Using the National Institutes of Health Test  
April 4, 2005
- 309 Supplemental Assay Method for the Titration of Canine Parainfluenza Virus in Vero Cell Culture  
June 6, 2005



- 320 Supplemental Assay Method for Titration of Feline *Chlamydia psittaci* in Embryonated Chicken Eggs  
March 5, 2001
- 321 Supplemental Assay Method for Quantitating the GP70 Antigen of Feline Leukemia Virus Veterinary Vaccines  
June 6, 2005
- 322 Supplemental Assay Method for Determination of the Specific Viral Antigen Content in Inactivated Canine Coronavirus Vaccines  
June 6, 2005
- 323 Supplemental Assay Method for Titration of Canine Distemper Virus in Vero Cell Culture  
June 6, 2005

400 Series

- 405 Supplemental Assay Method for Detecting Lymphoid Leukosis Biocontamination by the COFAL Test  
June 26, 2000
- 406 Supplemental Assay Method for Titration of Monovalent, Cell Associated Marek's Disease Vaccines of Serotypes 1, 2, or 3  
April 4, 2005
- 407 Supplemental Assay Method for Titration of Marek's Serotype 3 (Herpesvirus of Turkeys Strain FC-126), Lyophilized  
April 4, 2005
- 408 Supplemental Assay Method for Titrating Tissue Culture Adapted Vaccine Strains of Infectious Bursal Disease Virus  
April 4, 2005

409 Supplemental Assay Method for Titrating the Fractions of Combination Avian Encephalomyelitis/Pox Vaccine  
April 4, 2005

411 Supplemental Assay Method for the Titration of Newcastle Disease Vaccine, Infectious Bronchitis Vaccine, and Combination Newcastle Disease-Infectious Bronchitis Vaccine in Chicken Embryos  
April 4, 2005

500 Series

500 Supplemental Assay Method for the Determination of Phenol in Veterinary Biologics (pullorum antigen, *Mycoplasma synoviae* antigen, and *Mycoplasma gallisepticum* antigen)  
December 13, 2005

502 Supplemental Assay Method for the Determination of Residual Moisture in Veterinary Biologics Products  
October 20, 2004

504 Supplemental Assay Method for the Manual Determination of Protein Content of Veterinary Biologics (Biuret)  
April 4, 2005

509 Supplemental Assay Method for the Mikrozone Electrophoretic Separation of Blood Fractions Used in Veterinary Biologics  
April 4, 2005

510 Supplemental Assay Method for the Manual Determination of Formaldehyde in Veterinary Biologics (Schiff Test)  
April 4, 2005

512 Supplemental Assay Method for the Determination of Formaldehyde in Veterinary Biologics (Ferric Chloride Test)  
April 4, 2005

513 Supplemental Assay Method for the Determination of Protein and Phenol in PPD (Purified Protein Derivative Produced from Cultures of *Mycobacterium bovis* Strain AN-5) Bovis Tuberculin  
April 4, 2005

514 Supplemental Assay Method for the Determination of Hydrogen Ion Concentration, Total Nitrogen, TCA Nitrogen, Phenol and Clarity in Intradermic (Filtrate Produced From Cultures of Pn, C, and Dt Strains of *Mycobacterium tuberculosis*) Tuberculin  
April 4, 2005

600 Series

600 Supplemental Assay Method for Purity, Potency and Dissociation of *Brucella abortus* Vaccine, Strain 19  
June 19, 2000

601 Supplemental Assay Method for Potency Testing of Erysipelas Antiserum in Mice  
March 19, 1999

602 Supplemental Assay Method for Evaluation of Salmonella Pullorum Antigens  
May 18, 2000

603 Supplemental Assay Method for Potency Testing of Fowl Cholera (*Pasteurella multocida*) Bacterins, Type 3  
June 1, 2006

604 Supplemental Assay Method for the Evaluation of Koch's Old Tuberculin  
March 22, 1999

605 Supplemental Assay Method for Potency Testing of Erysipelas Bacterins in Swine  
May 25, 1982



- 617 Supplemental Assay Method for Potency Assay of *Leptospira interrogans*  
Serovar *grippotyphosa* Bacterins  
July 1, 1999
- 620 Supplemental Assay Method for Potency Testing Enterotoxigenic (K99 Pilus)  
*Escherichia coli* Bacterins  
June 20, 2006
- 621 Supplemental Assay Method for Potency Testing Enterotoxigenic (K88 Pilus)  
*Escherichia coli* Bacterins  
May 31, 2006
- 622 Supplemental Assay Method for Potency Testing Enterotoxigenic (987P Pilus)  
*Escherichia coli* Bacterins  
May 31, 2006
- 623 Supplemental Assay Method for Potency Testing Enterotoxigenic (F41 Pilus)  
*Escherichia coli* Bacterins  
June 6, 2006
- 624 Supplemental Assay Method for *in vitro* Potency Testing of *Leptospira*  
*interrogans* Serovar *pomona* Bacterins  
July 17, 2006
- 625 Supplemental Assay Method for *in vitro* Potency Testing of *Leptospira*  
*interrogans* Serovar *canicola* Bacterins  
July 17, 2006
- 626 Supplemental Assay Method for *in vitro* Potency Testing of *Leptospira*  
*interrogans* Serovar *grippotyphosa* Bacterins  
July 17, 2006
- 627 Supplemental Assay Method for *in vitro* Potency Testing of *Leptospira*  
*interrogans* Serovar *icterohaemorrhagiae* Bacterins  
July 17, 2006

- 630 Supplemental Assay Method for Potency Testing of Fowl Cholera (*Pasteurella multocida*) Bacterins, Type 4  
June 1, 2006
- 631 Supplemental Assay Method for Potency Testing of *Salmonella typhimurium* Bacterins  
June 19, 2006
- 632 Supplemental Assay Method for Potency Testing of *Salmonella choleraesuis* Bacterins  
June 20, 2006
- 633 Supplemental Assay Method for Potency Testing of *Salmonella dublin* Bacterins  
June 13, 2006
- 634 Supplemental Assay Method for Potency Testing of *Pasteurella multocida* Bacterins of Bovine Origin  
June 20, 2006
- 635 Supplemental Assay Method for Potency Testing of *Pasteurella multocida* Bacterins of Porcine Origin  
June 20, 2006
- 636 Supplemental Assay Method for the Evaluation of Tuberculin Purified Protein Derivative (PPD)  
May 22, 2000

800 Series

- 801 Supplemental Assay Method for Examination of Porcine Cells for Porcine Parvovirus Contamination  
September 16, 1983

802 Supplemental Assay Method for Identification of Vaccine Strains of Newcastle Disease Virus

December 16, 1998

803 Supplemental Assay Method for Examination of Trypsin Solutions for Porcine Parvovirus Contamination

June 15, 1983

900 Series

900 Supplemental Assay Method for Testing Growth Promoting Qualities of Fluid Thioglycollate Medium and Soybean-Casein Digest Medium using *Bacillus subtilis* Spores and *Candida krusei* as the Indicator Organisms

March 22, 1999

901 Supplemental Assay Method for Testing Growth-Promoting Qualities of Fluid Thioglycollate Medium with Beef Extract Using *Clostridium chauvoei* Spores as the Indicator Organism

March 3, 1999

902 Supplemental Assay Method for Testing Growth Promoting Qualities of Brain Heart Infusion Agar Using *Bacillus subtilis* Spores and *Candida krusei* as Indicator Organisms

July 13, 1999

903 Supplemental Assay Method for Testing for Preservative Interference with Sterility Tests

November 4, 1999

904 Supplemental Assay Method to Test for Bactericidal Activity of Diluents Used with Live Bacterial Vaccines

November 5, 1999

