

United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 600

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

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

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

1. Introduction

This Supplemental Assay Method (SAM) describes the test procedure used to determine the purity, dissociation and potency (viability) of *Brucella abortus* Vaccine (Strain 19), as prescribed in the Code of Federal Regulations, Title 9 (9 CFR), Part 113.65. Purity is determined by inoculating dextrose Andrades broth and thioglycollate broth; dissociation by inoculating potato agar plates; and potency by inoculating tryptose agar plates after diluting in 1% peptone water.

2. Materials

2.1 Equipment/instrumentation

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

- 2.1.1 Vortex mixer
- 2.1.2 Colony counter
- 2.1.3 Inoculum spreader
- 2.1.4 Disposable syringes and needles - appropriate sizes
- 2.1.5 Sterile disposable pipettes - appropriate sizes
- 2.1.6 Small disposable bottles
- 2.1.7 Pipetting aid
- 2.1.8 35°- 37°C incubator
- 2.1.9 Class II Biosafety cabinet
- 2.1.10 Lab coat or sterile sleevesgloves, and protective eyewear
- 2.1.11 Sterile gauze pads, 4 x 4-inch
- 2.1.12 Test tube rack
- 2.1.13 Sharpee™ container

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2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

2.2.1 Dextrose Andrades broth (**Appendix I**) - National Centers for Animal Health (NCAH) Media #10141

2.2.2 Tryptose agar (**Appendix II**) - NCAH Media #10093

2.2.3 Thioglycollate broth (**Appendix III**) - NCAH Media #10135

2.2.4 Potato agar (**Appendix IV**) - NCAH Media #10452

2.2.5 1% peptone saline solution (**Appendix V**) - NCAH Media #10138

2.2.6 Crystal violet solution (**Appendix VI**) - NCAH Media #30270

2.2.7 *B. abortus*, Strain 19 reference culture - National Veterinary Services Laboratories (NVSL) Diagnostic Bacteriology Laboratory (DBL) reagent code #15

2.2.8 Chemical disinfectant

2.2.9 70% ethyl alcohol

2.2.10 Sterile water in serum vials

3. Preparation for the Test

3.1 Personnel qualifications/training

Personnel performing the test 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, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn the laminar-flow hood on 30 minutes before use and turn off after use.

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3.2.2 Monitor the incubator, freezers and coolers daily for temperature.

3.3 Preparation of samples/reagents/control procedures

3.3.1 Allow the samples and reference cultures to warm to room temperature before rehydrating to the appropriate volume.

3.3.2 Negative and Positive Controls: Incubate 1 each of uninoculated tryptose and potato agar plates and 1 each of uninoculated dextrose Andrades and thioglycollate broth tubes with the test sample plates and tubes as negative controls. Dilute the *B. abortus* reference culture (positive control) and plate the same as the test samples.

3.3.3 Store plates used for making counts at refrigerator temperature. Allow the plates to be used for counts to dry under a biosafety cabinet before use. At the time of use, plates are no more than 30 days old.

4. Performance of the Test

4.1 Number all tubes, bottles, and plates.

4.2 Disinfect the top of the vials with 70% ethyl alcohol-soaked gauze pads. Reconstitute the desiccated products with enclosed sterile diluent, using the appropriate size syringe and needle. Mix until the vaccine cake is well dissolved.

4.3 Using a 10-mL syringe, remove approximately 10 mL of the vaccine from the vial of product. Place 0.2 mL each in 1 tube of dextrose Andrades broth and 1 tube thioglycollate broth. Place 0.1 mL onto a plate of potato agar. Transfer the rest of the product in the syringe to a sterile test tube.

4.4 With an inoculating loop, streak the 0.1 mL inoculum on the potato agar plate for bacterial isolation of individual colonies.

4.5 Mix the vaccine in the sterile test tube with a vortex mixer. Transfer 1.0 mL of the vaccine from the tube to a dilution bottle containing 99 mL of 1% peptone water using a sterile pipette. Mix the dilution bottle well by inversion.

4.6 Transfer 1.0 mL from the first 1% peptone water dilution bottle (10^{-2}) to a second bottle containing 99 mL of 1% peptone water (10^{-4}). Mix the dilution bottle well by inversion.

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- 4.7** Transfer 1.0 mL from the second (10^{-4}) 1% peptone water dilution bottle to a third dilution bottle containing 99 mL of 1% peptone water (10^{-6}). Mix the dilution bottle well by inversion.
- 4.8** Transfer 10.0 mL from the third (10^{-6}) 1% peptone water dilution bottle to a fourth dilution bottle containing 40 mL of 1% peptone water (10^{-7}). Mix the dilution bottle well by inversion.
- 4.9** Plate 0.1 mL of the fourth dilution bottle onto each of 4 tryptose agar plates using a pipette.
- 4.10** Spread the inoculum over the surface of the plates using a sterile inoculum spreader.
- 4.11** Repeat **Sections 4.3 through 4.10** with the second vial of the serial being tested.
- 4.12** Repeat **Sections 4.3 through 4.10** with 1 reference or positive control vial of *B. abortus*.
- 4.13** Invert all the agar plates (tryptose and potato agar) and incubate them and the dextrose Andrades and thioglycollate broths at 35°- 37°C for 4 days.
- 4.14** Negative (media) controls of both broths and agars are incubated along with the test.
- 4.15** Flood the plates of potato agar with crystal violet stain (see **Appendix VI**) for 20 seconds after incubation. Remove stain from plates by using a pipette with a pipetting device and place the stain in a bottle containing disinfectant. Observe the plates with a colony counter or dissecting microscope.
- 4.16** After incubation, average the colony forming units (CFU) for the 4 tryptose agar plates (see **Section 4.9**) and divide by 10, which will give billion (B) CFU per dose (CFU/D) per vial. Determine the mean CFU/D for the number of vials tested.

5. Interpretation of the Test Results

5.1 Purity testing

- 5.1.1** A serial is considered satisfactory (SAT) for purity when, observed macroscopically, there is no abnormal growth or color change of the indicator in the dextrose Andrades broth and no abnormal growth characteristics in the thioglycollate broth, once compared to the reference control tubes.

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5.1.2 If abnormal growth is observed macroscopically in any tube, the serial or subserial may be retested using 4 new vaccine samples, provided that if the retest (RT) is not done, the serial or subserial is unsatisfactory (UNSAT) for purity. Gram stains are prepared from any abnormal growth for microscopic examination.

5.2 Potency testing

5.2.1 A serial is considered SAT for potency when the average count from vials is between 3 and 10 B CFU/D of live *B. abortus* organisms.

5.2.2 If on the initial test the CFU/D is above 10 B CFU/D, the serial or subserial may be retested using 4 new vaccine samples, provided that if the retest is not done, the serial or subserial is UNSAT. If on the RT, the average count of the 4 vaccine samples is above the required range, RT the serial or subserial every 3 weeks until the average count drops below 10 B CFU/D.

5.2.3 If on the initial test the CFU/D is below 3 B CFU/D, the serial or subserial is RT with 4 vials. If the CFU/D of the retested vials is between 3 and 10 B CFU/D, the serial is SAT. If the CFU/D of the retest is below 3 B CFU/D, the serial is UNSAT.

5.3 Dissociation testing

5.3.1 A serial is considered SAT for dissociation when colonies on potato agar do not take the stain and remain white, an indication of smooth colony type.

5.3.2 A serial is considered UNSAT for dissociation if there are > 5% rough colony type or > 15% undesirable colony types which have stained red or purple.

6. Report of Test Results

Report results of test(s) as described by standard operating procedures.

7. References

Code of Federal Regulations, Title 9, Part 113.25, U.S. Government Printing Office, Washington, DC.

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8. Summary of Revisions

Version .03

- The Bacteriology Section Leader has been updated.
- Minor edits throughout document.

Version .02

- Reviewed 01Dec15, but no revisions needed.

Version .01

This document was revised 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 document number has been updated from STSAM0600 to SAM 600.
- The Contact phone number has been updated.
- **2.1:** The Bunsen burner has been removed.
- **3.1:** Personnel qualifications have been clarified.
- **3.2.2/3.2.3:** The references to internal documents have been removed.
- **Appendix:** Additional storage conditions for media have been added.

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Appendices

Appendix I

NCAH Media #10141

Dextrose Andrades broth

Beef infusion (double strength)	500.0 mL
QH ₂ O	500.0 mL
Bacto peptone	10.0 g
Sodium chloride	5.0 g

Mix and adjust pH to 7.4 with 10% sodium hydroxide (NaOH). Autoclave 30 minutes in flask at 121°C. Filter add:

1% dextrose	10.0 g
Regular Andrades indicator (0.5% stock solution)	10.0 mL

Read just pH to 7.4 with 10% NaOH. Autoclave 12 minutes at 121°C. Store at 20°- 25°C for up to 30 days.

Appendix II

NCAH Media #10093

Tryptose agar

Tryptose agar	41.0 g
H ₂ O	1000.0 mL

Autoclave at 121°C for 25 minutes. Cool in waterbath at 56°C. Store at 2°- 5°C for up to 30 days.

Appendix III

NCAH Media #10135

Fluid thioglycollate medium

Fluid thioglycollate medium	29.5 g
QH ₂ O	1000.0 mL

Mix and heat to boiling. Autoclave 20 minutes at 121°C. Store at 20°- 25°C for up to 30 days.

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NCAH Media #10452

Potato agar (powder)

Potato infusion agar	49.0 g
Glycerol	20.0 mL
QH ₂ O	1000.0 mL

Stir and autoclave 20 minutes. Store at 2°- 5°C for up to 30 days.

Appendix V

NCAH Media #10138

Peptone solution 1% + 0.5% NaCl

Bacto peptone	10.0 g
NaCl	5.0 g
QH ₂ O	1000.0 mL

Autoclave for 20 minutes at 121°C. Store at 20°- 25°C for up to 30 days.

Appendix VI

NCAH Media #30270

Crystal violet ammonium oxalate (stock sol) DBL

Sol 1:

Crystal violet	2.0 g
Absolute ethanol	20.0 mL

Mix until dissolved.

Sol 2:

Ammonium oxalate	0.8 g
QH ₂ O	80.0 mL

Mix until dissolved, then mix Sol 1 and Sol 2 together to form a stock sol (should be stored in amber bottle at 20°- 25°C for up to 30 days). The stock sol is diluted 1:40 in distilled water just before use.