United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 911

Supplemental Assay Method for the Detection of *Salmonella* in Antibody Products

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Supplemental Assay Method for the Detection of *Salmonella* in Antibody Products

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1. Introduction

This Supplemental Assay Method (SAM) describes the test procedure used to detect *Salmonella* contamination in dried and liquid antibody products for oral administration, per title 9, *Code of Federal Regulations* (9 CFR), part 113.450(h)(2)(ii). This test procedure uses Brilliant green agar (BGA) to detect *Salmonella* contamination.

2. Materials

2.1 Equipment/instrumentation

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

2.1.1 Laminar-flow Class II biosafety cabinet (BSC)

2.1.2 Lab Armor® bead bath (set to 50°- 55°C)

2.1.3 Vortex mixer

2.1.4 30°- 35°C incubator

2.1.5 Analytical balance

2.1.6 Colony counter

2.2 Reagents/supplies

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

2.2.1 *Salmonella enterica* (American Type Culture Collection (ATCC) #13076) or equivalent positive control culture

2.2.2 Brilliant green agar (BGA) (**Appendix**), National Centers for Animal Health (NCAH) Media #10541 or as stated in the Outline of Production (OP) from the biologics manufacturer.

2.2.3 Sterile water

2.2.4 Glycerol diluent, 50%

2.2.5 Sterile pipettes, individually packaged

2.2.6 Sterile petri dishes, 100 x 15-mm

2.2.7 Sterile culture tubes

2.2.8 Lab coat or sterile sleeves and gloves

2.2.9 Spatulas

2.2.10 Weigh boats

2.2.11 Sterile wide mouth snap cap specimen containers

2.2.12 70% ethanol

2.2.13 4 x 4-inch sterile gauze pads

2.2.14 Pipetting aid

2.2.15 Micropipettors, 100-µL to 1.0-mL

2.2.16 Pipette tips, 100-µL to 1.0-mL

2.2.17 Sterile syringes with needles

2.2.18 Sarstedt vials, 2 mL

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, 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 Operate all equipment and instrumentation according to the manufacturer's instructions and maintain according to standard operating procedures (SOPs).

3.2.2 Turn on the BSC at the beginning of the work week and leave on all week.

3.2.3 Turn on the bead bath at least one day before use to allow the temperature to equilibrate.

3.2.4 Monitor temperature of incubators, freezers, coolers, and bead baths according to SOPs.

3.3 Preparation of reagents/control procedures

3.3.1 Warm the samples and reference culture to 20°- 25°C (room temperature) before rehydrating, if needed, to the volume listed on the vial or in the firm's OP. Thaw frozen products in the BSC immediately before testing.

3.3.2 *S. enterica* reference stock culture is prepared according to the manufacturer's instructions, suspended in 50% sterile glycerol, and mixed on a stir plate. Aliquots of 1.2 mL of prepared culture are dispensed into Sarstedt vials and stored at -75° ± 5°C. For each test session, inoculate a petri dish with 10-100 CFU/0.1 mL to serve as a positive control.

3.3.3 Glycerol diluent, 50%: The 50% glycerol diluent is prepared by mixing equal parts of glycerol and 0.85% NaCl solution. Place 300 mL of diluent in a 500-mL flask and sterilize at ≥121°C for 25 to 30 minutes or by following the manufacturer's recommendations. Store at 20°- 25°C (room temperature) for 1 year.

3.3.4 Negative/Technique Control: Inoculate one petri dish with 1 mL of the water that was used as a diluent in the testing session. If no diluent is used in the testing session, use sterile water packaged in serum vials as the inoculum. Pour 15-20 mL of 50°- 55°C molten BGA into each plate, swirl gently to mix and allow to solidify. Once the agar has hardened, invert the petri dish. Incubate this control plate with the serial test plates.

3.3.5 Preparation of the BGA medium: On the day of the test, melt the BGA in an autoclave for 30 minutes at 100°C. Place the bottles of melted media in the bead bath. Do not begin testing until the agar has cooled to at least 55°C. The temperature of the bead bath should be approximately 50°- 55°C when the agar is ready.

3.4 Preparation of the sample

3.4.1 At least ten containers of final product are required for *Salmonella* purity testing.

3.4.2 Samples are liquid or dried antibody products. When needed, sterile purified water in volumes specified on the product label or the diluent specified in the firm's OP is used for rehydrating samples.

3.4.3 Weigh samples of product on an analytical balance, if needed. Rehydrate samples of the product according to label or instructions from the firm's OP. Dissolve samples by stirring on a stir plate or shake with a vortex mixer. If sample does not readily go into solution after manipulating, allow the diluted sample to sit in a 30°- 35°C incubator for 1 hour (hr) ± 30 minutes (min).

3.4.4 Label 10 plates for each serial with the sample number or name and the container number.

4. Performance of the Test

4.1 Dress in a clean lab coat or sterile sleeves and gloves to perform purity testing.

4.2 Wipe down the interior surfaces of the BSC used for testing with 70% ethanol immediately prior to use and between testing each serial.

4.3 Place the necessary testing materials (micropipettors, pipet tips, 4 x 4-inch gauze squares, petri dishes, etc.) and the diluted (as stated in the firm's OP) sample into the BSC.

4.4 Mix the sample thoroughly. Dispense 1 mL of inoculum from one sample container into a petri dish.

4.5 Repeat **Section 4.4** for the other nine sample containers for this serial.

4.6 Dispense 15-20 mL of 50°- 55°C molten BGA medium into each of the 10 dishes for this serial. Swirl gently to mix and then allow the agar to cool in the BSC. Once the agar has hardened, invert the petri dishes and place the dishes in a 30°- 35°C incubator for 24 ± 4 hours (hrs).

4.7 Repeat **Sections 4.3** through **4.6** for the other serials of biologic to be tested.

4.8 Once all the test serials have been put on test, prepare the negative/technique controls for the testing session (see **Section 3.3.4**).

4.9 Once the purity portion of the test has been completed, prepare the positive control organism in an area that is separate and apart from the clean area where the purity test was conducted (see **Section 3.3.2**).

4.9.1 Inoculate a petri dish with 10-100 CFU/0.1 mL of *S. enterica*.

4.9.2 Dispense 15-20 mL of 50°- 55°C molten BGA medium into the plate. Swirl gently to mix and then allow the agar to cool in the BSC. Once the agar has hardened, invert the petri dish.

4.10 All plates are incubated at 30°- 35°C for 24 ± 4 hrs.

5. Interpretation of the Test Results

5.1 Criteria for a valid test:

5.1.1 There must be no growth in the negative/technique control and media control plates.

5.1.2 The positive control plate is examined for *Salmonella* characteristic colony growth and must contain a range of 10-100 CFU/0.1 mL. Characteristic growth is identified as red to pink-white colonies surrounded by a red zone in the medium.

5.1.3 If these criteria are not met, the test is considered invalid or a no test (NT). Products may be reported and released with a NT result if there is no reason to suspect an unsatisfactory (UNSAT) sterility result for that product.

5.2 After incubation, all plates are examined macroscopically for growth typical of *Salmonella*. If *Salmonella* characteristic colony growth (see **Section 5.1.2**) appears on the positive control plate and does not appear on any negative control plate, the test is valid.

5.3 If no *Salmonella* colonies are detected on any of the sample agar plates, the OP serial is determined to be negative for *Salmonella* contamination and is satisfactory (SAT). If *Salmonella* characteristic colony growth is observed on any of the sample agar plates, the test is considered inconclusive (INC), and one retest (RT; see **Section 5.5**) to rule out faulty technique, may be conducted. If a RT is not conducted within 21 days, the serial is UNSAT by the first test.

5.4 If growth is observed on the negative or media control plate(s), or no characteristic growth is observed on the positive control plate, the test is a NT and may be repeated without prejudice.

5.5 If a RT is ordered, double the number of samples used in the first test are required. If *Salmonella* characteristic colony growth is observed on any of the RT plates, the serial is UNSAT.

6. Record and Report of Test Results

Record and report results of the test(s) according to SOPs.

7. References

Title 9, *Code of Federal Regulations*, part 113.450(h)(2)(ii), U.S. Government Printing Office, Washington, DC.

Standard methods for the examination of dairy products. 1992, 16th Edition, Robert T. Marshall editor. American Public Health Association, Washington, D.C.

8. Summary of Revisions

Version .02

- The coversheet has been updated and document reformatted.

Appendix

Brilliant Green Agar (BGA) – National Centers for Animal Health (NCAH) Media #10541

Brilliant Green Agar Base	58.0 g
QH_20	1000.0 mL

Autoclave 20 minutes at 121°C. Store at 2°- 5°C for up to 6 months.