

United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 914

Supplemental Assay Method for the Detection of Fungi in Antibody Products

Date: May 2, 2017
Number: SAM 914.01
Supersedes: New
Standard Requirement: 9 CFR 113.450(h)(2)(iii)
Contact: Sophia G. Campbell, (515) 337-7489

Approvals:

/s/Larry R. Ludemann Date: 12May17
Larry R. Ludemann, Section Leader
Bacteriology

/s/Paul J. Hauer Date: 01Jun17
Paul J. Hauer, Director
Policy, Evaluation, and Licensing
Center for Veterinary Biologics

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

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Entered into CVB Quality Management System by: <u>/s/Linda S. Snavely</u> <u>06Jun17</u> Linda S. Snavely Date Quality Management Program Assistant
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Supplemental Assay Method for the Detection of Fungi in Antibody Products

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Supplemental Assay Method for the Detection of Fungi in Antibody Products

1. Introduction

This Supplemental Assay Method (SAM) describes the test procedure used to detect fungi contamination in dried and liquid antibody products for oral administration, per title 9, *Code of Federal Regulations* (9 CFR), part 113.450(h)(2)(iii). This test procedure uses Acidified Potato Dextrose agar (APDA) to detect fungi 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°- 60°C)

2.1.3 Vortex mixer

2.1.4 20°-25°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 *Issatchenkia orientalis* (American Type Culture Collection (ATCC) #6258) or equivalent organism

2.2.2 Acidified Potato Dextrose agar (APDA) (**Appendix I**), National Centers for Animal Health (NCAH) Media #50102 or as stated in the Outline of Production (OP) from the biologics manufacturer.

2.2.3 Tartaric Acid (TA) (**Appendix II**), NCAH Media #50103

Note: Tartaric acid is a corrosive. Wear appropriate personal protective equipment when handling.

2.2.4 Sterile water

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- 2.2.5 0.15M Phosphate Buffered Saline with 12% sucrose (PBS w/ 12% sucrose)
- 2.2.6 Sterile pipettes, individually packaged
- 2.2.7 Sterile petri dishes, 100 x 15-mm
- 2.2.8 Sterile culture tubes
- 2.2.9 Lab coat or sterile sleeves and gloves
- 2.2.10 Spatulas
- 2.2.11 Weigh boats
- 2.2.12 Sterile wide mouth snap cap specimen containers
- 2.2.13 70% ethanol
- 2.2.14 4 x 4-inch sterile gauze pads
- 2.2.15 Pipetting aid
- 2.2.16 Micropipettors, 100- μ L to 1.0-mL
- 2.2.17 Pipette tips, 100- μ L to 1.0-mL
- 2.2.18 Sterile syringes with needles
- 2.2.19 Glass serum bottles, 2.0-mL
- 2.2.20 Rubber seals and metal caps for serum bottles

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**.

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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 *I. orientalis* reference stock culture is prepared according to the manufacturer's instructions, suspended in PBS with 12% sucrose, mixed on a stir plate, 1.0 mL of culture dispensed into 2.0-mL glass serum vials, loosely capped, lyophilized, crimped, labeled, and then stored at $-75^{\circ} \pm 5^{\circ}\text{C}$. For each test session, inoculate a petri dish with 10-100 CFU/0.1 mL to serve as a positive control.

3.3.3 PBS w/ 12% sucrose: The PBS w/ 12% sucrose is prepared by mixing 440 mL of PBS and 60 g sucrose, dispensing 300 mL into a 500-mL flask, and sterilizing at 121°C for 20 minutes (min) or by following the manufacturer's recommendations. Store at 20°- 25°C (room temperature) for 1 year.

3.3.4 Preparation of the APDA medium: On the day of the test, melt the APDA 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 57°C. The temperature of the bead bath should be approximately 57°C when the agar is ready. Just prior to using the APDA medium, add 1 mL of TA/100 mL of molten APDA medium.

3.3.5 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 52°- 57°C molten APDA 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.

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3.4 Preparation of the sample

3.4.1 At least ten containers of final product are required for fungi 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 52°- 57°C molten APDA 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 20°- 25°C incubator for 5 days.

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 3.3.5**).

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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 *I. orientalis*.

4.9.2 Dispense 15-20 mL of 52°- 57°C molten APDA 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 20°- 25°C for 5 days.

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 fungi characteristic growth and must contain a range of 10-100 CFU/0.1 mL. Characteristic growths are identified as creamy to white colony growth for yeasts and filamentous colony growth of various colors (like yellow, orange, red, etc.) for molds.

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 typical fungi growth. If fungi 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 fungi colonies are detected on any of the sample agar plates, the serial is determined to be negative for fungi contamination and is satisfactory (SAT). If fungi 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.

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5.5 If a RT is ordered, double the number of samples used in the first test are required. If fungi 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

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

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

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Appendices

Appendix I

Acidified Potato Dextrose Agar (APDA) – National Centers for Animal Health (NCAH)
Media #50102

Potato Dextrose Agar	39.0 g
QH ₂ O	1000.0 mL
Tartaric acid 10% Solution (NCAH Media #50103)	10.0 mL

For plates: Combine agar and water. Mix well. Autoclave 15 minutes at 121°C. Cool in a 52°- 57°C water bath for a minimum of 1 hour. Aseptically add tartaric acid solution and mix well. Dispense. Store at 2°- 5°C for up to 6 months.

For bottles: Combine agar and water. Mix well. Bring to a boil and dispense. Autoclave 15 minutes at 121°C. Prepare 100 mL of tartaric acid solution and deliver separately with bottles. Store at 2°- 5°C for up to 6 months.

Appendix II

Tartaric Acid (TA) – NCAH Media #50103

Tartaric Acid	10.0 g
QH ₂ O	100.0 mL

Note: Tartaric acid is a corrosive. Wear appropriate personal protective equipment when handling.

In a low flow hood, combine acid and water. Mix well. Filter sterilize. Aseptically dispense. Label with a corrosive sticker. Store at 20°- 25°C (room temperature) for up to a year.