United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 902

Supplemental Assay Method for Testing Growth-Promoting Qualities of Brain Heart Infusion Agar using *Bacillus subtilis* Spores and *Issatchenkia orientalis* as Indicator Organisms

Date: **February 25, 2015**

Number: SAM 902.04

Supersedes: SAM 902.03, February 1, 2012

Standard Requirement: 9 CFR 113.25

Contact: Sophia G. Campbell, (515) 337-7489

Approvals:

/s/Larry R. Ludemann Date: 09Mar15
Larry R. Ludemann, Section Leader
Bacteriology

/s/Byron E. Rippke Date: 10Mar15
Byron E. Rippke, Acting Director
Policy, Evaluation, and Licensing
Center for Veterinary Biologics

/s/Rebecca L.W. Hyde Date: 13Mar15
Rebecca L.W. Hyde, Section Leader
Quality Management
Center for Veterinary Biologics

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

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may be suitable.

UNCONTROLLED COPY

Supplemental Assay Method for Testing Growth-Promoting Qualities of Brain Heart Infusion Agar using *Bacillus subtilis* Spores and *Issatchenkia orientalis* as Indicator Organisms

Table of Contents

UNCONTROLLED COPY

Supplemental Assay Method for Testing Growth-Promoting Qualities of Brain Heart Infusion Agar using *Bacillus subtilis* Spores and *Issatchenkia orientalis* as Indicator Organisms

1. Introduction

This Supplemental Assay Method (SAM) describes testing Brain Heart Infusion Agar (BHIA) for growth promoting qualities, as required in title 9, *Code of Federal Regulations* (9 CFR), part 113.25(b). Each lot of media that is used for sterility testing of biological products (9 CFR 113.26 through 113.27) must be tested for growth promoting qualities.

2. Materials

2.1 Equipment/instrumentation

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

2.1.1 30º- 35ºC incubator

2.1.2 20º- 25ºC incubator

2.1.3 Thermo Scientific Finnpippette electronic pipette

2.1.4 Laminar-flow Class II biosafety cabinet (BSC)

2.1.5 Vortex mixer

2.1.6 Lab Armor® bead bath (set to 55°- 60°C)

2.2 Reagents/supplies

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

2.2.1 *Bacillus subtilis* (American Type Culture Collection (ATCC) #6633) or equivalent organism as specified in the current United States Pharmacopoeia (USP)

2.2.2 *Issatchenkia orientalis* (ATCC #6258) or equivalent organism as specified in the current USP

2.2.3 Brain-Heart Infusion Agar (BHIA), National Centers for Animal Health (NCAH) Media #10204 (**Appendix I**)

UNCONTROLLED COPY

2.2.4 Soybean-Casein Digest Medium (SCDM), NCAH Media #10423 (**Appendix II**)

Note: Trypticase Soy Broth (TSB) and SCDM are the same media formulation from different media companies.

2.2.5 Penicillinase Concentrate, 10,000,000 Kinetic (Kersey) units/mL (BBL catalog number 211898)

2.2.6 Petri dishes, 100 x 15-mm

2.2.7 70% ethanol

2.2.8 4 x 4-inch sterile gauze pads

2.2.9 Sterile pipettes

2.2.10 Sterile pipet tips, 100-1000 µL

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 Monitor temperature of incubators and waterbath according to SOPs.

3.2.3 Turn on the biosafety cabinet at least 30 minutes before starting work.

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

3.3 Preparation of reagents/control procedures

3.3.1 *Bacillus subtilis* stock culture is prepared according to the manufacturer's instructions and titrated to determine colony forming unit (CFU) concentration.

3.3.2 *Issatchenkia orientalis* stock culture is prepared according to the manufacturer's instructions and titrated to CFU concentration.

3.3.3 A working solution of 100,000 Kinetic (Kersey) units of penicillinase/mL is prepared by adding 1.0 mL of the penicillinase concentrate (**Section 2.2.5**) into 99.0 mL of sterile water.

3.3.4 Preparation of the BHIA medium: On the day of the test, melt the BHIA 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 60°C. The temperature of the bead bath should be approximately 60°C when the agar is ready. The BHIA supplemented medium is prepared by adding the penicillinase working solution to the BHIA to yield 500 Kinetic (Kersey) units of penicillinase/mL of medium, just prior to pouring the medium into plates.

4. Performance of the Test

Test each batch of BHIA for growth promoting qualities using both *B. subtilis* and *I. orientalis* as indicator organisms.

4.1 Thaw the frozen vials of indicator organism stock culture in the BSC. Rehydrate lyophilized stock cultures with SCDM according to the reagent data sheet. Mix stock cultures thoroughly by vortexing immediately prior to use.

4.2 Prepare dilutions of the indicator organism stock cultures according to the reagent data sheet specifications. Mix the dilutions by vortex mixing.

4.3 For each indicator organism, use a Finnpippette electronic pipette with a sterile tip, to dispense into each of four sterile 15 x 100-mm petri dishes the volume of inoculum that will yield approximately100 CFU.

4.4 Pour 20-25 mL of BHIA supplemented with penicillinase into each of two plates containing *B. subtilis*. Pour 20-25 mL of the new batch of BHIA without penicillinase into each of the other two plates containing *B. subtilis*.

4.5 Repeat **Section 4.4** with the *I. orientalis* culture.

UNCONTROLLED COPY

4.6 Incubate the four petri dishes containing *B. subtilis* culture at 30°- 35°C for 7 days. Incubate the four petri dishes containing the *I. orientalis* culture at 20°- 25°C for 14 days.

4.7 On day 7, count the number of CFUs in the *B. subtilis* plates and record the counts on the test worksheet. Average the counts for the two plates of BHIA supplemented with penicillinase separately from the two plates of BHIA without penicillinase.

4.8 On day 14, count the number of CFUs in the *I. orientalis* plates and record the counts on the test worksheet. Average the counts for the two plates of BHIA supplemented with penicillinase separately from the two plates of BHIA without penicillinase.

5. Interpretation of the Test Results

5.1 If the average colony count for each organism in plates supplemented with penicillinase is within ± 20% of the average colony count of that same organism in plates without penicillinase, the growth-promoting quality of that batch of BHIA medium is satisfactory (SAT) and may be used for sterility testing.

5.2 If the average colony count for each organism in plates supplemented with penicillinase is not within ± 20% of the average colony count of that same organism in BHIA without penicillinase, the growth-promoting quality of that batch of medium is unsatisfactory (UNSAT) and is not acceptable for use in sterility testing.

5.3 If any sterility tests have been conducted with unsatisfactory batches of BHIA, those tests must be reported as no tests (NT) and repeated with a new satisfactory batch of BHIA media.

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 113.25, U.S. Government Printing Office, Washington, DC.

7.2 The U.S. Pharmacopoeia, 1985, Vol. 21, pp 1151-1160, Mack Publishing Co., Easton, PA.

UNCONTROLLED COPY

7.3 Kurtzman, C. P., C. J. Robnett, and E. Basehoar-Powers. 2008. Phylogenetic relationships among species of Pichia, Issatchenkia and Williopsis determined from multigene sequence analysis, and the proposal of Barnettozyma gen. nov., Lindnera gen. nov. and Wickerhamomyces gen. nov. FEMS Yeast Res 8:939-54.

8. Summary of Revisions

Version .04

- The Bacteriology Section Leader has been updated.
- Deleted formerly *Candida krusei* from this document.
- Sections 2-3 have been updated and rewritten for clarity.

Version .03

- The contact information has been updated.
- *Candida krusei* has been changed to the new name *Issatchenkia orientalis* and this document was updated to reflect the new name.
- **3.3:** Renamed and updated to reflect current practices.
- **4:** This section has been updated to reflect current practices.
- **5:** The interpretation of test results has been clarified.
- **7.3:** Reference added about *Issatchenkia orientalis*.
- **Appendices:** Updated to include current media numbers.
- **Appendix III**: Control limits are established according to standard operating procedures, so this section has been removed from this document.

Version .02

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 changed from STSAM0902 to SAM 902.

Supplemental Assay Method for Testing Growth-Promoting Qualities of Brain Heart Infusion Agar using *Bacillus subtilis* Spores and *Issatchenkia orientalis* as Indicator Organisms

- The Contact has been changed from Gerald Christianson to Sophia G. Campbell.
- **1:** Information has been added to clarify the testing purpose.
- **2.1.5:** Petri dishes have been added to the equipment list.
- **2.1.6:** The class of biosafety cabinet to be used has been added.
- **2.2:** The list of reagents/supplies has been updated.
- **3.1:** Personnel qualifications have been clarified
- **3.2.2:** This section has been revised to indicate additional equipment monitored.
- **4.1/4.2:** These sections have been revised to clarify the procedures followed in testing.
- **5:** The test interpretations have been clarified.
- **Appendices I & II:** Media storage conditions have been added.
- The statistical tolerances table has been moved from the body of the document to **Appendix III**.

UNCONTROLLED COPY

Supplemental Assay Method for Testing Growth-Promoting Qualities of Brain Heart Infusion Agar using *Bacillus subtilis* Spores and *Issatchenkia orientalis* as Indicator Organisms

Appendices - Media Formulations

Appendix I

Brain Heart Infusion Agar (BHIA) – NCAH Media #10204

Brain Heart Infusion Agar	52 g
QH_2O	1000 mL

Autoclave 20 minutes at 121ºC. Store at 2°- 5°C for no longer than 3 months.

Appendix II

Trypticase Soy Broth (TSB) or Soybean-Casein Digest Medium (SCDM) – NCAH Media #10423

Trypticase Soy Broth	30 g
QH_2O	1000 mL

Autoclave 20 minutes at 121ºC. Store at 20°- 25°C for no longer than 3 months.

TSB and SCDM are two names for the same media formulation from different media companies.

UNCONTROLLED COPY