United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 900

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

Date: **March 19, 2015**

Number: SAM 900.04

Supersedes: SAM 900.03, March 7, 2012

Standard Requirement: 9 CFR 113.25

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

Approvals:

/s/Larry R. Ludemann Date: 02Apr15
Larry R. Ludemann, Section Leader
Bacteriology

/s/Byron E. Rippke Date: 03Apr15
Byron E. Rippke, Acting Director
Policy, Evaluation, and Licensing
Center for Veterinary Biologics

/s/Rebecca L.W. Hyde Date: 03Apr15
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

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

Table of Contents

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

1. Introduction

This Supplemental Assay Method (SAM) describes testing Fluid Thioglycollate Medium (FTM) and Soybean-Casein Digest Medium (SCDM) 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 – 113.27) must be tested for growth promoting qualities.

Note: Trypticase Soy Broth (TSB) and SCDM are the same media formulation and the terms are used interchangeably throughout this document.

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 HandyStep® electronic pipette

2.1.4 Laminar-flow Class II biosafety cabinet (BSC)

2.1.5 Vortex mixer

2.2 Reagents/supplies

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

2.2.1 *Bacillus subtilis* (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 Soybean-Casein Digest Medium (SCDM) (National Centers for Animal Health (NCAH) Media #10423) (**Appendix I**)

2.2.4 Fluid Thioglycollate Medium (FTM) (NCAH Media #10135) (**Appendix II**)

2.2.5 Sterile pipettes

2.2.6 BRAND PD-Tip™ Syringe Tips

2.2.7 4 x 4-inch sterile gauze pads

2.2.8 Tubes, 25 x 200-mm, with sterile closures

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 according to SOPs.

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

3.3 Preparation of reagent/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.

4. Performance of the Test

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

4.1 Thaw the frozen vials of indicator organism stock cultures 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 using a vortex mixer.

4.3 Prepare a sufficient volume of each working dilution for each indicator organism (i.e., 25-30 milliliter (mL) volume of each of the working dilutions).

4.4 Use a HandyStep pipette with a sterile syringe tip to dispense 1.0 mL of the higher working dilution of *B. subtilis* into each of ten 25 x 200-mm tubes containing 40.0 mL of SCDM. Change syringe tip and use to dispense 1.0 mL of the higher working dilution into each of ten 25 x 200-mm tubes containing 40.0 mL of FTM.

4.5 Change syringe tip and use to dispense 1.0 mL of the next lower working dilution of *B. subtilis* into each of ten 25 x 200-mm tubes containing 40.0 mL of SCDM. Change syringe tip and use to dispense 1.0 mL of the lower working dilution into each of ten 25 x 200-mm tubes containing 40.0 mL of FTM.

4.6 Repeat **Sections 4.1 through 4.5** for the *I. orientalis* indicator organism.

4.7 Incubate all tubes (40) containing the *B. subtilis* culture at 30º- 35ºC and observe for growth of the organism throughout a 7-day incubation period.

4.8 Incubate all tubes (40) containing the *I. orientalis* culture at 20º- 25ºC and observe for growth of the organism throughout a 14-day incubation period.

5. Interpretation of the Test Results

Growth is expected in at least 8 or more tubes inoculated with the lowest working dilution of each indicator organism and in greater than zero, but less than 8 tubes inoculated with the next higher working dilution of each indicator organism.

5.1 If at least 8 of the tubes inoculated with the lower working dilution of a stock culture contain growth, the growth promoting quality of that medium is satisfactory (SAT).

5.2 If less than 8 tubes inoculated with the lower working dilution of a stock culture have growth, then the growth promoting qualities of the media are in question and the test must be repeated.

5.3 If the media's growth promoting properties are still in question after a retest, the media must not be used and all tests conducted with this media lot must be considered no tests (NT).

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.

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.
- Section 1 was rewritten for clarity.

Version .03

This document was revised to clarify practices currently in use at the Center for Veterinary Biologics and to provide additional detail. One significant change was made that might impact

the designation of the serial/lot final disposition of the test; the following changes were made to the document:

- 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.
- The table of contents has been updated.
- **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. This might change the outcome of the sterility test and could impact CVB's overall disposition of the serial/lot on test.
- **7.3:** Reference added about *Issatchenkia orientalis*.
- **Appendices:** Updated to include current media numbers.

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 Contact has been changed from Gerald Christianson to Sophia Campbell.
- **1:** Clarification on the use of this SAM and the media designations have been added.
- **2:** The list of equipment/instrumentation has been updated to reflect current procedures.
- **3.3.3**: The status of a valid Gram stain for *B. subtilis* has been clarified.
- **3.3.5:** A filtration step to remove beads has been added.
- **3.4.2:** The status of a valid Gram stain for *C. krusei* has been clarified.

- **4.1:** This section has been rewritten to provide further clarification of current procedures and to add an explanation on titration of each new lot of indicator organisms.

- **4.2:** This section has been rewritten to provide further clarification of current procedures.

- **5**: The interpretation of test results has been clarified.

- **Appendices:** The storage conditions have been added.

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

Appendices – Media Formulations

Appendix I

NCAH Media #10423

Trypticase Soy Broth (TSB) or Soybean-Casein Digest Medium (SCDM)

Trypticase Soy Broth	30 g
QH_2O	1000 mL

Autoclave 20 minutes at 121ºC. Media is stored at 20°- 25°C for up to 3 months.

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

Appendix II

NCAH Media #10135

Fluid Thioglycollate Medium (BBL)

Fluid Thioglycollate Medium	29.5 g
QH_2O	1000 mL

Mix and heat to boiling. Autoclave 20 minutes at 121°C. Media is stored at 20°-25°C for up to 3 months.