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United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 901

Supplemental Assay Method for Testing Growth-Promoting Qualities of Fluid Thioglycollate Medium with Beef Extract Using *Clostridium chauvoei* Spores as the Indicator Organism

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Standard Requirement: 9 CFR 113.25

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1. Introduction

This Supplemental Assay Method (SAM) describes testing Fluid Thioglycollate Medium with Beef Extract (FTM/BE) 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.

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 HandyStep® electronic pipette

2.1.3 Laminar-flow Class II biosafety cabinet (BSC)

2.1.4 Vortex mixer

2.2 Reagents/supplies

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

2.2.1 *Clostridium chauvoei* spores or equivalent organism as specified in the current United States Pharmacopoeia (USP)

2.2.2 Fluid Thioglycollate Medium with 0.5% Beef Extract (FTM/BE), National Centers for Animal Health (NCAH) Media #10227 (**Appendix**)

2.2.3 Sterile pipettes

2.2.4 BRAND PD-Tip™ Syringe Tips

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

2.2.6 4 x 4-inch sterile gauze pads

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

C. chauvoei stock culture is prepared according to the manufacturer's instructions.

4. Performance of the Test

Test each batch of FTM/BE for growth promoting qualities using *C. chauvoei* as the indicator organism.

4.1 Thaw a frozen vial of *C. chauvoei* stock culture in the BSC. Mix stock culture thoroughly by vortexing immediately prior to use.

4.2 Prepare dilutions of the *C. chauvoei* stock culture according to the reagent data sheet specifications. Mix the dilutions using a vortex mixer.

4.3 Prepare a sufficient volume of each working dilution of *C. chauvoei* (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 *C. chauvoei* into each of ten 25 x 200-mm tubes containing 40.0 mL of FTM/BE. 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/BE.

4.5 Incubate all tubes (20) at 30º- 35ºC and observe for growth of the organism throughout the 14-day incubation period.

5. Interpretation of the Test Results

Growth is expected in at least 9 or more tubes inoculated with the lowest working dilution of the indicator organism and in greater than zero, but less than 9 tubes inoculated with the next higher working dilution of the indicator organism.

5.1 If at least 9 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 9 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 a 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.

8. Summary of Revisions

Version .05

- Updated cover page.

Version .04

- The Bacteriology Section Leader has been updated.
- Sections 1-3 have been updated.

Version .03

- The contact information has been updated.
- 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.
- **Appendix:** 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 document number has been changed from STSAM0901 to SAM 901.
- The Contact has been changed from Gerald Christianson to Sophia G. Campbell.
- **1:** Information to clarify the testing purpose has been added.
- **2.1.4:** Sterile glass tubes have been added to the equipment list.
- **2.1.5**: The class of biosafety cabinet to be used has been added.
- **2.1.8**: An anaerobic growth chamber for use in *C. chauveoi* propagation and spore harvest has been added.
- **2.2**: The list of reagents/supplies has been updated.
- **3.1:** Personnel qualifications have been clarified.
- **3.3:** Information on the growth of *C. chauveoi* culture and harvest of spore suspension has been added.

- **4.1/4.2:** These sections have been revised to clarify the procedures followed in testing.

- **5:** The test interpretations have been clarified.

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

- **Appendix III:** A temperature range has been added for the storage of Normal Saline.

- **Appendix IV:** This section has been added to provide the media recipe for beef infusion agar medium.

- **Appendix V**: This section has been added to provide the media recipe for 0.015M phosphate buffered saline pH 6.9.

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Appendix

NCAH Media #10227

Fluid Thioglycollate with Beef Extract

Fluid Thioglycollate Medium	29.5 g
QH_2O	1000 mL

Heat and add:

0.5% Beef Extract (Difco)	5 g

Bring to a boil and dispense. Autoclave 20 minutes at 121ºC. Store at 20°- 25°C for no longer than 3 months.