

FLUID THIOGLYCOLLATE MEDIUM (7137)

Intended Use

Fluid Thioglycollate Medium is used for sterility testing. This formula conforms to the US Pharmacopeia (USP).¹

Product Summary and Explanation

Quastel and Stephenson² found that the presence of small amounts of a compound containing an –SH group (cystein, thioglycollic acid and glutathione) permitted “aerobic” growth of *Clostridium sporogenes*. Falk, Bucca and Simmons,³ discovered the advantages of using small quantities of agar in detecting contaminants during sterility testing. The value of a small amount of agar and a reducing substance was demonstrated by Brewer.⁴

Fluid Thioglycollate Medium is also referred to as Thioglycollate Medium, and abbreviated FTM. Fluid Thioglycollate Medium is prepared according to the formula specified in the FDA Bacteriological Analytical Manual (BAM),⁵ and the AOAC Official Methods of Analysis⁶ for the examination of food, and sporicidal effects of disinfectants. FTM is recommended for sterility checks on banked blood,⁷ and blood cultures.⁸

Principles of the Procedure

Fluid Thioglycollate Medium supports the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Casein and Yeast Extract. Sodium Thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides that can be toxic to some organisms. The sulfhydryl groups (-SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material containing heavy metals.

Resazurin is the oxidation indicator. In the oxidized state, resazurin turns pink. In the reduced state resazurin is colorless. Dextrose is included in this formula to enhance organism growth. Sodium Chloride maintains the osmotic balance of the medium. The requirement for a sealed environment is eliminated with the addition of Agar, which retards dispersion of CO₂, diffusion of oxygen, and reducing substances.⁹

Formula / Liter

Enzymatic Digest of Casein	15 g
Yeast Extract	5 g
Dextrose	5.5 g
L-Cystine	0.5 g
Sodium Chloride.....	2.5 g
Sodium Thioglycollate	0.5 g
Resazurin	0.001 g
Agar.....	0.75 g

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, skin, and respiratory system.

Directions

1. Suspend 29.8 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

NOTE: Unless used on the same day of preparation, the prepared tubes should be boiled (with caps loose) for 3 - 5 minutes and cooled before use.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is hazy, yellow in color, and may have a slight precipitate with a light to medium red upper layer. If the light to medium red layer is greater than 10% of the tube, the medium may be restored once by heating in a steam bath until the light to medium red color disappears.

Expected Cultural Response: Cultural response at an inoculum level of 10 - 300 cfu's at 35 ± 2°C for 18 to 48 hours and examined for growth.

Microorganism	Expected Results
<i>Clostridium novyi</i> ATCC® 7659	good to excellent growth
<i>Clostridium perfringens</i> ATCC® 13124	good to excellent growth
<i>Neisseria meningitidis</i> ATCC® 13090	poor to excellent growth
<i>Streptococcus pyogenes</i> ATCC® 19615	poor to excellent growth

The organisms listed are the minimum that should be used for quality control testing.

USP Growth Promotion Testing: Cultural response at an inoculum level of 10-100 cfu's incubated at the specified temperatures and conditions for up to 7 days and examined for growth.

Microorganism	Expected Results
<i>Bacteroides vulgatus</i> ATCC® 8482	growth
<i>Bacillus subtilis</i> ATCC® 6633	growth
<i>Candida albicans</i> ATCC® 10231	growth
<i>Micrococcus luteus</i> ATCC® 9341	growth

Test Procedure

Refer to appropriate references for specific procedures using Fluid Thioglycollate Medium.

Results

Typically growth is visually observed in the medium. Gram-negative bacilli usually grow diffusely, Gram-positive cocci exhibit puff-ball type growth, and strict aerobes, such as pseudomonads and yeast, tend to grow in a thin layer on the surface of the broth.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date. The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Fluid Thioglycollate Medium	Code No.	7137A	500 g
		7137B	2 kg
		7137C	10 kg

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.
2. **Quastel and Stephenson.** 1926. General biological products standards. Fed. Regist. **21**:6109.12.
3. **Falk, C. R., H. Bucca, and M. P. Simmons.** 1939. A comparative study of the use of varying concentrations of agar in the the medium used to detect contaminants in biological products, J. Bacteriol. **37**:121-131.
4. **Brewer, J. H.** 1940. Clear liquid mediums for the "aerobic" cultivation of anaerobes. J. Amer. Med. Assoc. **115**:598-600.
5. **Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
6. **Association of Official Analytical Chemists.** 1995. Official Methods of Analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
7. **Federal Register.** 1992. Additional standard for human blood and blood products. Fed Regist. **21**:640.2.17.
8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D.C.
9. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification maintenance of medical bacteria, vol.1, p. 755-762. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.