



Fluid Thioglycollate Medium

ME009

Fluid Thioglycollate Media are used for sterility testing of biologicals and for cultivation of aerobic and anaerobic organisms and microaerophiles in accordance with European Pharmacopoeia

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	15.000
Yeast extract	5.000
Glucose monohydrate	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750
pH after sterilization (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.25 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml Water R/purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 25°C and store in a cool dark place preferably below 25°C.

Note: If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink colour disappears.

Principle And Interpretation

Brewer (1) formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes by adding a reducing agent and small amount of agar. The European Pharmacopoeia (2), USP (3), BP (4) and AOAC (5) have recommended the media for sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials.

Glucose monohydrate, pancreatic digest of casein, yeast extract provide necessary nutrients and growth factors necessary for bacterial multiplication. Sodium thioglycollate, 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 which can be toxic to some organisms. The sulphahydril groups (-SH) of these compounds neutralizes the antibacterial effect of mercurial preservatives and other heavy metal compounds which exert a bacteriostatic effect in the materials under examination. Any increase in the oxygen content is indicated by a colour change of redox indicator, resazurin to pink (6,7,8). The small amount of agar helps in maintaining low redox potential for stabilizing the medium (9).

In sterility checking, when the sample contains preservatives, it is recommended to dilute the sample with this broth to reduce the toxicity and enhance the growth of contaminants, if any.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light straw coloured clear to slightly opalescent solution with upper 10% or less medium pink on standing.

Reaction

After sterilization, reaction of 2.92% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Growth Promotion Test

As per European Pharmacopoeia

Stability test

Light yellow coloured clear solution without any precipitation sedimentation at room temperature for 7 days

Cultural response

Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days.

Cultural Response

Organism	Inoculum (CFU)	Growth
When incubated anaerobically		
<i>Clostridium sporogenes</i> ATCC 19404	50 -100	luxuriant
<i>Clostridium sporogenes</i> ATCC 11437	50 -100	luxuriant
<i>Clostridium sporogenes</i> NBRC 14293	50 -100	luxuriant
<i>Clostridium perfringens</i> ATCC 13124	50 -100	luxuriant
<i>Bacteroides fragilis</i> ATCC 23745	50 -100	luxuriant
<i>Bacteroides vulgatus</i> ATCC 8482	50 -100	luxuriant
When incubated aerobically		
<i>Staphylococcus aureus</i> ATCC25923	50 -100	luxuriant
<i>Staphylococcus aureus</i> ATCC 6538	50 -100	luxuriant
<i>Pseudomonas aeruginosa</i> ATCC 27853	50 -100	luxuriant
<i>Pseudomonas aeruginosa</i> ATCC 9027	50 -100	luxuriant
<i>Micrococcus luteus</i> ATCC 9341	50 -100	luxuriant
<i>Streptococcus pneumoniae</i> ATCC 6305	50 -100	luxuriant
<i>Escherichia coli</i> ATCC 25922	50 -100	luxuriant
<i>Escherichia coli</i> ATCC 8739	50 -100	luxuriant
<i>Escherichia coli</i> NCTC 9002	50 -100	luxuriant
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant
<i>Salmonella Abony</i> NCTC 6017	50 -100	luxuriant
<i>Bacillus subtilis</i> ATCC 6633	50 -100	luxuriant

Storage and Shelf Life

Store below 30°C and the prepared medium at room temperature and away from light. Use before expiry date on the label.

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2. European Pharmacopoeia, 2012. European Department, for the Quality of Medicines.
3. U.S. Pharmacopoeia, 2012, United States Pharmacopoeia Convention, Inc., Rockville, MD.
4. British Pharmacopoeia, 2012, The Stationery office British Pharmacopoeia.
5. Williams. (Ed.), 2008, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., AOAC, Washington, D.C.
6. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.

7. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287.
8. Portwood, 1944, J. Bact., 48:255.
9. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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