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TECHNICAL PRODUCT INFORMATION

Fluid Thioglycollate Medium w/Indicator (USP Formulation)
Fluid Thioglycollate Medium w/o Indicator

INTENDED USE:
Fluid Thioglycollate Medium is a general purpose liquid enrichment medium used in qualitative procedures for the sterility test and for the isolation and cultivation of aerobes, anaerobes and microaerophiles that are not excessively fastidious. In clinical microbiology, it may be used as an enrichment medium for clinical specimens.

Thioglycollate Medium w/o Indicator is an enriched general purpose medium used for cultivating and detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives when the oxidation-reduction indicator is not present or required. Thioglycollate Medium w/o Indicator is the medium of choice for diagnostic testing, where lack of an indicator avoids possible toxicity to organisms.[4]

HISTORY/SUMMARY:
Quastel and Stephenson found the presence of small amounts of a compound containing an –SH group (cysteine, thioglycollic acid, and glutathione) permitted "aerobic" growth of Clostridium sporogenes.[1] Falk, Bucca, and Simmons discovered the advantages of using small quantities of agar in detecting contaminants during sterility testing. [2] Brewer demonstrated the value of combining a small amount of agar and a reducing substance in this medium. With the addition of a small amount of agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate .[3] Later it was confirmed by Nungster, Hood and Warren and Portwood the bacterial effect of mercurial compounds was neutralized by sodium thioglycollate.[4, 5]

In 1941 the National Institute of Health recommended the use of two different formulations of Thioglycollate Media for sterility testing. Both formulations contained 0.1% Sodium Thioglycollate as a reducing agent and a dilution of methylene blue as a pH indicator, and include other components.

Later Christiensen reported the toxic effects of the indicator on some organisms. The investigator suggested the use of resazurin in the Thioglycollate as an alternative to methylene blue. He also reported that the sodium chloride concentrations used in this medium were toxic to some organisms frequently found in sterility testing, as well as potassium phosphate. Sodium thioglycollate in concentrations not greater than 0.05% was only slightly toxic and adequately neutralized the mercurial preservatives.

Thioglycollate medium formulation contains a small amount (0.07%) of agar which impedes diffusion of oxygen.

The United States Public Health Services recommend this Thioglycollate formulation for the sterility testing of clear fluid biologics. The formula is prepared following specifications for Thioglycollate medium given by the U.S. Pharmacopeia and the National Formulary.
Thioglycollate medium NIH also called USP Alternate Thioglycollate Medium is an alternative formulation used for sterility testing of biologics that are turbid and could not be cultured in a viscous medium. This formula conforms to the specifications given in the U.S. Pharmacopeia and the National Formulary[1].

PRINCIPLES:
This medium is capable of supporting good growth of a great variety of organisms, including strict anaerobes, without incubation in an anaerobic atmosphere. The reducing properties of sodium thioglycollate are used in this medium to maintain adequate oxidation-reduction potential for anaerobic growth. This substance also shows the advantage of inactivating a small concentration of mercurial preservatives present in many biological formulations.

Glucose, peptone, and yeast extract provide the growth factors necessary for bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Sodium thioglycollate and L-cystine are reducing agents that prevent the accumulation of peroxides which are lethal to some microorganisms. 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. Agar eliminates the need for seals because it retards dispersion of CO_2, diffusion of oxygen, and reducing substances, thereby maintaining anaerobiosis in the lower depths of the medium.[4, 5] Due to its agar content, Fluid Thioglycollate Medium often appears slightly opaque.

In Fluid Thioglycollate Medium with indicator, resazurin is an oxidation-reduction indicator, being pink when oxidized (as oxygen is absorbed into the medium) and colorless when reduced. If the upper 30% layer of broth turns pink (oxidized resazurin), the medium should be heated in boiling water or a steam bath, to drive off the absorbed oxygen. Caps should be loose during this process and tightened immediately after. Medium should not be reheated more than once.

FORMULA: **Ingredients per liter of purified water:**

Thioglycollate Medium w/o Indicator Final pH 7.0 ± 0.2	
Pancreatic Digest of Casein	17.0 g
Enzymatic Digest of Soybean Meal	3.0 g
Dextrose	5.5 g
Sodium Thioglycollate	0.5 g
Sodium Chloride	2.5 g
L-Cystine	0.25 g
Agar	0.75 g

Thioglycollate with Indicator Final pH 7.1 ± 0.2	
Pancreatic Digest of Casein	15.0 g
Yeast Extract	5.0 g
Dextrose (anhydrous)	5.0 g
Sodium Thioglycollate	0.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Resazurin	0.001 g
Agar	0.75 g

QUALITY CONTROL BOTTLES (per USP[2])

CONTROL ORGANISM	**RESULTS**
ATCC# 9027 Pseudomonas aeruginosa	Growth in 24 – 72 hours
ATCC# 6538 Staphylococcus aureus	Growth in 24 – 72 hours
ATCC# 11437 Clostridium sporogenes	Growth in 24 – 72 hours
ATCC# 6633 Bacillus subtilis	Growth in 24 – 72 hours
ATCC# 10231 Candida albicans	Growth in 24 – 72 hours
ATCC# 16404 Aspergillus brasiliensis	Growth in 24 – 72 hours

QUALITY CONTROL TUBES (per CLSI) Media is classified as Exempt in CLSI M22-A3.[3]

CONTROL ORGANISM	**RESULTS**
ATCC# 25285 Bacteroides fragilis	Growth at 48 hours
ATCC# 25923 *Staphylococcus aureus*	Growth at 48 hours

PRECAUTIONS:
Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. These products are for In Vitro Diagnostic Use or Laboratory Use as indicated by product label.

STORAGE:
This media may be stored at 2-30°C (preferably in the dark).

REFERENCES:

1. Kurtin, American Journal of Clinical Pathology, 30:239. 1958
2. Current Edition of U.S. Pharmacopeia
3. CLSI, M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard 3rd Edition, June 2004
4. Difco & BBL Manual, Manual of Culture Media, 2003 Pages 554-560
5. Acumedia 7160 Product Information Sheet Rev 5, 2014