THIOGLYCOLLATE BROTH

INTENDED USE

Fluid Thioglycollate Medium (FTM) is used for the sterility testing of biologics and for the cultivation of anaerobes, aerobes and microaerophiles.

SUMMARY AND EXPLANATION

Quastel and Stephenson[1] found that the presence of a small amount of a compound containing an –SH group (cysteine, thioglycollic acid, glutathione) permitted "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.

Falk, Bucca and Simmons[2] pointed out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. The value of combining a small amount of agar and a reducing substance was demonstrated by Brewer.[3] Brewer's experiments revealed that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[4] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Nungester, Hood and Warren[5] and Portwood[6] confirmed the neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate. Incorporation of casein peptone was introduced by Vera.[7] Malin and Finn[8] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate. In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[9] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[10]

Fluid Thioglycollate Medium is recommended in the FDA Bacteriological Analytical Manual (BAM)[11] and the Official Methods of Analysis of AOAC International[12] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[13] It is one of the media recommended in the USP for use in sterility testing of articles purporting to be sterile; these formulations meet the requirements of the USP growth promotion test.[14]

PRINCIPLE

Dextrose, peptone, L-cystine and yeast extract provide the growth factors necessary for bacterial replication. Sodium chloride provides essential ions. Sodium thioglycollate is a reducing agent that prevents the accumulation of peroxides which are lethal to some microorganisms. The L-cystine is also a reducing agent, since it contains sulfhydryl groups which inactivate heavy metal compounds and maintain a low

redox potential, thereby supporting anaerobiosis. Methylene blue is an indicator of the level of oxidation/reduction in the medium; increased oxidation raises the Eh, causing the methylene blue indicator to become green. Resazurin is an oxidation-reduction indicator, being pink when oxidized and colorless when reduced. The small amount of agar assists in the maintenance of a low redox potential by stabilizing the medium against convection currents, thereby maintaining anaerobiosis in the lower depths of the medium.

REAGENTS (FORMULA)

Pancreatic Digest of Casein	15.0	g
Yeast Extract	5.0	g
Dextrose	5.5	g
Sodium Chloride	2.5	g
L-Cystine	0.5	g
Sodium Thioglycollate	0.5	g
Agar	0.75	g
Resazurin	1.0	mg
Deionized Water	1000.0	ml

PROCEDURE

Follow the procedures outlined in the references and, where applicable, in product package inserts.

EXPECTED RESULTS

After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized layer.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Clostridium novyi ATCC 7659	30-35°C for 18-48 hours	Growth
Clostridium perfringens ATCC 13124	30-35°C for 18-48 hours	Growth
Staphylococcus aureus ATCC 25923	30-35°C for 18-48 hours	Growth

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