PRODUCT SPECIFICATION SHEET

Brewer Thioglycollate Medium (DM620)

Intended Use

Brewer Thioglycollate Medium (DM620) is recommended for testing sterility of biological products and for isolation of a wide variety of aerobic and anaerobic organisms.

Product Summary and Explanation

Brewer thioglycollate medium is prepared as per the original formula of Brewer.[1, 2] Quastel and Stephenson[3] noticed that the presence of a small amount of a compound containing an –SH group (cysteine, thioglycollic acid, glutathione) allowed "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth. Falk, Bucca and Simmons[4] brought out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. Brewer[1] demonstrated the value of combining a small amount of agar and a reducing substance. Brewer's experiments revealed that anaerobes grew equally well, in a liquid medium containing 0.05% agar, irrespective of presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[5] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate was confirmed by Nungester, Hood and Warren[6] and Portwood.[7] Vera[8] introduced incorporation of casein peptone. Malin and Finn[9] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate.

Principles of the Procedure

Brewer Thioglycollate Medium, Modified contains highly nutritious highly nutritious proteose peptone and beef infusion, which support luxuriant growth of even fastidious bacteria. Dextrose is the fermentable carbohydrate and energy source. Sodium thioglycollate aids in creating anaerobic condition and also neutralizes toxicity of mercurial compounds if present in the inoculum of the test sample. Sodium chloride maintains the osmotic equilibrium of the medium. Dipotassium phosphate serves as a buffering agent. Very small amount of agar present maintains anaerobic conditions at the bottom of the broth. Methylene blue indicates oxygen content of the medium by exhibiting bluish-green colour to the medium in presence of oxygen. In an uninoculated medium bluish green colour at the top indicates presence of oxygen in that part. The medium contains more thioglycollate and is recommended for sterility testing procedures. Organisms that ferment dextrose and lower the pH to critical levels may not survive in this medium after growth has taken place.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	10.00
Beef, infusion form	500.00
Dextrose	5.00
Sodium chloride	5.00
Dipotassium phosphate	2.00
Sodium thioglycollate	0.50
Methylene blue	0.002
Agar	0.50
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

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

3. If more than the upper one third layer acquires bluish-green colour (absorbs oxygen), the dissolved oxygen can be removed by heating the medium in free flowing steam for 5-10 minutes or in a water bath until the green colour disappears, and the prepared medium should be stored in the dark till use.
4. Do not reheat the media more than once; continued reheating gives rise to toxicity.

Directions

1. Suspend 40.5 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in tubes or in suitable containers as desired.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear to slightly opalescent fluid with upper 10% or less medium bluish green on standing
Reaction of 4.05% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours (*Clostridium* and *Bacteroides* species incubated anaerobically).

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Bacteroides melaninogenicus ATCC 25848*	50 - 100	good-luxuriant
2.	*Clostridium sporogenes ATCC 11437*	50 - 100	good-luxuriant
3.	*Streptococcus mitis ATCC 9895*	50 - 100	good-luxuriant
4.	*Streptococcus pyogenes ATCC 19615*	50 - 100	good-luxuriant
5.	*Bacteroides fragilis ATCC 25285*	50 - 100	good-luxuriant
6.	*Staphylococcus aureus ATCC 25923*	50 - 100	good-luxuriant

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

1. After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control.
2. 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.

Storage

Store the sealed bottle containing the dehydrated medium at 10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if 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.

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Limitations of the Procedure

1. Anaerobes can be overgrown by more rapidly growing facultative organisms.
2. If plating medium reveals no growth examine and Gram stain broth.
3. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Brewer Thioglycollate Medium
Product Code : DM620
Available Pack sizes : 100gm/ 500gm

References

1. Brewer. 1940. JAMA *115*:598.
2. Brewer J. H., 1940, J. Bacteriol., 39:10
3. Quastel and Stephenson. 1926. J. Biochem. *20*:1125.
4. Falk, Bucca and Simmons. 1939. J. Bacteriol. *37*:121.
5. Marshall, Ginnish and Luxen. 1940. Proc. Soc. Exp. Biol. Med. *43*:672.
6. Nungester, Hood and Warren. 1943. Proc. Soc. Exp. Biol. Med. 52:287.
7. Portwood. 1944. J. Bacteriol. *48*:255.
8. Vera. 1944. J. Bacteriol. *47*:59.
9. Malin and Finn. 1957. J. Bacteriol. *62*:349.

Further Information

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DM620PSS,QAD/FR/024,Rev.00/01.01.2018

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