PRODUCT SPECIFICATION SHEET

Buffered Glucose Broth (DM142BS)

Intended use
Buffered Glucose Broth (DM142BS) is recommended for performing Methyl Red and Voges Proskauer tests in differentiation of coliaerogenes group.

Product Summary and Explanation
In 1915, Clark and Lubs[1] demonstrated that the colon-aerogenes family of bacteria could be divided in to two groups based on their action in a peptone and dextrose medium. When tested with pH indicator Methyl red, the "coli" group produced high acidity while the "aerogenes" group produced a less acid reaction. The test to detect high acid end product is known as the Methyl Red (MR) test. This test distinguishes those organisms able to form large amounts of acid from glucose so that the pH falls below 4.4 produce low pH level. Voges and Proskauer[2] described a red fluorescent coloration which appeared after the addition of potassium hydroxide to cultures of certain organisms in glucose medium. The red colour produced by the addition of potassium hydroxide to cultures is due to the ability of organisms to produce a neutral product acetoin (acetyl methyl carbinol) from dextrose.[3] The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl which reacts with creatine to produce a red colour and this test was known as VP test. This formulation is also recommended by BIS[4] and ISO committee[5] for the detection of coli-aerogenes group. For the detection of *E. coli, Vibrio parahaemolyticus* and *Bacillus cereus* responsible for food poisoning a slightly modified formulation (DM142BS) is recommended by BIS.[6,7,8] To test *V.parahaemolyticus* for VP, addition of 2-3% Sodium chloride to the medium is required.

Principles of the Procedure
Buffered Glucose Broth contains peptic digest of animal tissue which provides are carbon, nitrogen, and vitamin used for general growth requirements. Dextrose is the fermentable carbohydrate. Dipotassium phosphate is a buffering agent.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.00
Dextrose	5.00
Dipotassium phospate	5.00
Final pH: 7.5 ± 0.1 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.

Directions
1. Suspend 15 grams of the medium in 1000 ml of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Distribute in test tubes in 3 ml amounts or as desired.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream coloured homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear solution without any precipitate
Reaction of 1.5% Solution	pH : 7.5 ± 0.1 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 30-32°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	MR Test	VP Test
1.	*Bacillus cereus ATCC 10876*	50-100	good-luxuriant	negative reaction, yellow colour	positive reaction, eosin pink / red colour within 2-5 minutes
2.	*Enterobacter aerogenes ATCC 13048*	50-100	good-luxuriant	negative reaction, yellow colour	positive reaction, eosin pink / red colour within 2-5 minutes
3.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	positive reaction, bright red colour	negative reaction, no colour change
4.	*Klebsiella pneumoniae ATCC 23357*	50-100	good-luxuriant	negative reaction, yellow colour	positive reaction, eosin pink / red colour within 2-5 minutes
5.	*Salmonella Typhi ATCC 6539*	50-100	good-luxuriant	positive reaction, bright red colour	negative reaction, no colour change
6.	*Vibrio parahaemolyticus ATCC 17802*	50-100	poor	negative reaction, yellow colour	negative reaction, no colour change

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

Test Procedure

1. Methyl Red (MR) test is performed after maximum of 5 days of incubation at 30°C.[9]
2. Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours.[10]
3. A variety of other tests have been suggested by Werkman,[11] OMeara[12] Levine, Epstein and Voughn[13] and Voughn, Mitchell and Levine.[9]
4. Werkmans Test : Add 2 drops of a 2% solution of ferric chloride to 50 ml culture and 5 ml of 10% sodium hydroxide. Shake the tube to mix well. Stable copper colour developing in a few minutes is positive reaction.[5]
5. OMeara Test : Add of 25 mg of solid creatine to 5ml culture and then add 5 ml concentrated (40%) sodium hydroxide. Red colour development in a few minutes after shaking the tube well, is a positive reaction.[5]
6. Levine, Epstein and Voughn[13] modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide.
7. Voughn, Mitchell and Levine[9] recommended the method of Barritt[14] as, addition of 1 ml of 40% potassium hydroxide and 3 ml of 5% a - naphthol in absolute ethanol to 5 ml culture. Positive test is indicated by eosine pink colour within 2-5 minutes.
8. Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and test procedures for interpretation of results.

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.

Limitations of the Procedure

1. The MR-VP reactions are only part of the tests required to identify organisms.
2. Each laboratory should standardize on the inoculums density, volume of broth and the test container size.
3. MR test require a minimum incubation of 48 hours before the pH indicator is added.
4. When using Barritt's reagent add a-naphthol first and KOH second; do not reverse this order.
5. Vaughn et al.[9] warned of false positive VP reactions if the completed tests are left standing for over an hour.

Packaging
Product Name : Buffered Glucose Broth
Product Code : DM142BS
Available Pack sizes : 100gm / 500gm

References

1. Clark and Lubs, 1915, J. Inf. Dis., 17 : 160.
2. Voges and Proskauer, 1898, Zeit, Hyg., 28 : 20. ,,
3. MacFaddin, J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. Bureau of Indian Standards, IS : 5887 (Part - III) 1976.
5. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 6597.
6. Bureau of Indian Standards, IS : 5887 (Part I) 1976, reaffirmed 1986.
7. Bureau of Indian Standards, IS : 5887 (Part IV) 1976.
8. Bureau of Indian Standards, IS : 5887 (Part - V) 1976, reaffirmed 1986.
9. Vaughn, Mitchell and Levine, 1939, J. Am. Water Works Association, 31:993.
10. Kallas, Chinn and Coulter, 1931, J. Bact., 22 : 125.
11. Werkman, 1930, J. Bact., 20 : 121.
12. OMeara, 1931, J. Path. Bacteriol., 34 : 401.
13. Levine, Epstein and Voughn, 1934, Am. J. of Publ. Health, 24 : 505.
14. Barritt, 1936, J. Path. Bacteriol., 42 : 441.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall', Thane (W) – 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com