Minerals Modified Glutamate Medium

LAB 80A & LAB 80B

Description
This medium was developed for use with the Most Probable Numbers Technique (M.P.N.) for the enumeration of coliforms in water supplies. The medium is an improved version of the chemically defined glutamic acid medium described by Gray in 1964. The product is supplied in two parts because it has been shown that separating the sodium glutamate from the base improves its stability.

Typical Formula	**g/litre**
LAB 80a (double strength)	
Lactose	20.0
Sodium formate	0.5
L-Cystine	0.04
L(-) Aspartic acid	0.048
L(+) Arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate ($MgSO_4.7H_20$)	0.2
Ferric ammonium citrate	0.02
Calcium chloride ($CaCl_2.2H_2O$)	0.02
Dipotassium hydrogen phosphate	1.8
Bromocresol purple	0.02
LAB 80b	
Glutamic acid (sodium salt)	12.7

Method for reconstitution
Double strength: Dissolve 22.7 grams of base medium (LAB 80a) together with 12.7 grams of sodium glutamate (LAB 80b) in 1 litre of deionised water containing 5 grams of ammonium chloride eg BDH cat no. 27149. Dispense 10ml and 50ml volumes into tubes with inverted Durham tube.

Single strength: Dissolve in 11.35 grams of base medium (LAB 80a) together with 6.35 grams of sodium glutamate (LAB 80b) in 1 litre of distilled water containing 2.5 grams ammonium chloride. Dispense 5ml volumes into tubes with inverted Durham tubes.

Sterilise by autoclaving for 10 minutes at 115°C, alternatively heat to 100°C for 30 minutes on three successive days.

Appearance: Purple, clear solution.

pH: 6.7 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation: Use the Most Probable Number technique. With 10ml and 50ml of sample add to equal volumes of double strength medium. With 1ml volumes of sample add to 5ml of single strength medium. Ensure the Durham tube is free of bubbles.

Incubation: 37°C for 18-24 hours aerobically.

Interpretation Tubes showing the production of acid (medium turns yellow) and gas in the Durham's tube are considered presumptive positive. Each presumptive positive tube should be subcultured to Brilliant Green Bile Broth LAB 51 with Durham tube and incubated at 44°C for 24 hours and examined for gas production. A tube of Tryptone Water LAB 129 should also be inoculated and incubated at 44°C for 24 hours for the production of indole. The production at 44°C of gas from lactose and the formation of indole are evidence of *E. coli.*

References
Gray, R.D. (1964). An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J. Hyg. Camb. 62: 495-508.

PHLS Water Sub-Committee. (1958). A comparison between MacConkey broth and Glutamic acid media for the detection of coliform organisms in water. J. Hyg. Camb. 56: 377-388.

PHLS Standing Committee on Bacteriological Examination of Water Supplies. (1968). Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of Coliform organisms in water. J. Hyg. Camb. 66: 67-87.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.