

1. Prepare milk dilutions of 1/10, 1/100, 1/1,000 in 1/4-strength Ringer's solution. Use this inoculum within 15 minutes.
2. Pour Plates: Pipette 1 mL of each dilution into Petri dishes. Add 10-12 mL of molten Milk Agar, cooled to 45°C, and mix thoroughly.
Spread Plates: Spread 1 mL of milk dilution over the surface of the solidified medium in a Petri dish.
3. Incubate at 30 ± 2°C for 72 hours.

Expected Results

Select plates containing 10-300 colonies. Results are expressed as colonies per mL of product tested.

Proteolytic psychrotrophic colonies may be enhanced by flooding the plates with a solution of 1% hydrochloric acid or 10% acetic acid. Pour off the excess acid solution and count the colonies surrounded by clear zones.

References

1. Methods of microbiological examination for dairy purposes. Diluents, media and apparatus and their preparation and sterilisation. BS4285, Sec. 1.2.
2. International Dairy Federation. 1993. Milk and milk products – preparation of samples and dilutions for microbiological examination. Standard 122A: 1988.
3. Klose. 1968. Susswaren. 14:778.
4. Ministry of Health. 1937. Bacteriological tests for graded milk. Memo 139/Foods. H.M.S.O., London, England.

Availability

Difco™ Milk Agar

Cat. No. 218591 Dehydrated – 500 g

Minerals Modified Glutamate Broth

Intended Use

Minerals Modified Glutamate Broth is used for enumerating coliform organisms in water.

Summary and Explanation

Gray¹ described a simple formate-lactose-glutamate medium that could be used as an alternative to MacConkey Broth for the presumptive identification of coliform bacteria in water. Gray's original medium gave fewer false positive results than MacConkey Broth, was suitable for use at 44°C and gave low volumes of gas.

The medium was improved² by the addition of ammonium chloride that, by replacing ammonium lactate, resulted in a doubling of the gas volume. The addition of B-complex vitamins, certain amino acids and magnesium ions resulted in an increased rate of fermentation. Comparative trials of the modified glutamate medium and MacConkey Broth³ with chlorinated and unchlorinated waters showed that Gray's Minerals Modified Glutamate Broth gave significantly higher numbers of positive results (acid and gas production) for coliform organisms and *Escherichia coli*. This was especially apparent after 48 hours of incubation but was also clearly seen with unchlorinated water samples after only 24 hours incubation. For chlorinated water samples, results with the two media were comparable. After 18-24 hours incubation, Minerals Modified Glutamate Medium gave significantly fewer false positive reactions. *Clostridium perfringens*, a common cause of false positive reactions in MacConkey media, is unable to grow in a minimal-glutamate based medium.

A major feature of Minerals Modified Glutamate Medium is its superiority in initiating growth of *Escherichia coli* after exposure to chlorine when incubated for 48 hours. In view of the known resistance to chlorination of some viruses, the ability to isolate coliform bacteria that survive marginal chlorination provides an additional safety factor in water treatment.

In a comparison to Lauryl Tryptose Lactose Broth⁴, Minerals Modified Glutamate Medium gave superior isolation of *Escherichia coli* after 48 hours incubation by the multiple tube method, especially in waters containing small numbers of organisms. Minerals Modified Glutamate Medium is the medium of choice for the detection of fecal contamination in chlorinated drinking water supplies in Great Britain.⁴

Abbiss et al.⁵ compared Minerals Modified Glutamate Medium and three other enrichment broths for the enumeration of coliform organisms present in soft cheese, cooked meat and patè. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

User Quality Control

Identity Specifications

Difco™ Minerals Modified Glutamate Broth

Dehydrated Appearance:	Off-white to beige, free flowing, homogeneous.
Solution:	1.77% solution with 0.25% ammonium chloride, soluble in purified water on gentle warming. Solution is purple, clear.
Prepared Appearance:	Purple, clear.
Reaction of 1.77% Solution at 25°C:	pH 6.7 ± 0.1 (containing 0.25 g of ammonium chloride per 100 mL)

Cultural Response

Difco™ Minerals Modified Glutamate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
<i>Enterobacter aerogenes</i>	13048	30-300	Good	+	+
				(yellow)	
<i>Enterococcus faecalis</i>	19433	10 ³	None	–	–
<i>Escherichia coli</i>	25922	30-300	Good	+	+
				(yellow)	
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	30-300	Good	–	–

Minerals Modified Glutamate Broth has been used in the modified direct plate method for enumeration of *Escherichia coli* biotype 1 in foods.⁶ According to this method, 15 grams of agar are added per liter of single strength broth before autoclaving. The medium is poured in 12-15 mL amounts into sterile Petri dishes. This resuscitation agar is used for the recovery of damaged cells from frozen or dried foodstuffs.

Principles of the Procedure

Sodium glutamate and sodium formate are the basis of a defined minimal medium for the enumeration of coliform organisms in water. Lactose is the carbohydrate source in Minerals Modified Glutamate Broth. The addition of B-complex vitamins, certain amino acids and magnesium ions allows an increased rate of fermentation. Phosphate acts as a buffering agent. The addition of ammonium chloride allows increased gas production by the test organism. Bromocresol purple is present as a pH indicator.

Formula

Difco™ Minerals Modified Glutamate Broth

Approximate Formula* Per Liter

Sodium Glutamate	6.4	g
Lactose	10.0	g
Sodium Formate	0.25	g
L-Cystine	0.02	g
L(-) Aspartic Acid	24.0	mg
L(+) Arginine	0.02	g
Thiamine	1.0	mg
Nicotinic Acid	1.0	mg
Pantothenic Acid	1.0	mg
Magnesium Sulfate Heptahydrate	0.1	g
Ferric Ammonium Citrate	0.01	g
Calcium Chloride Dihydrate	0.01	g
Dipotassium Phosphate	0.9	g
Bromocresol Purple	0.01	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 17.7 g of the powder in 1 L of purified water (or in 500 mL to prepare a double strength medium).
2. Add 2.5 g of ammonium chloride.
3. Mix well with gentle heating to dissolve.
4. Distribute into tubes and place an inverted fermentation tube in each tube.
5. Autoclave at 115-116°C for 10 minutes.
6. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

The multiple tube method is used for the enumeration of *Escherichia coli* and coliform organisms using Minerals Modified Glutamate Broth.

For good quality water, inoculate the water sample into the medium in the following volumes:

1. 50 mL of sample into 50 mL of double-strength medium;
2. 5 × 10 mL of sample into 5 × 10 mL of double-strength medium.

For more polluted waters, inoculate the water sample into the medium in the following volumes:

1. 5 × 1 mL of sample into 5 × 5 mL of single-strength medium;
2. 5 × 1 mL of a 1:10 dilution of the sample into 5 × 5 mL of single-strength medium.

Incubate the tubes at 35 ± 2°C. Examine after 18-24 hours incubation and again at 48 hours.

Expected Results

All tubes demonstrating acid production, indicated by the medium turning yellow, and gas, either in the inverted fermentation vial or by effervescence on shaking, may be regarded as presumptive positive reactions. Each presumptive positive tube should be confirmed in Brilliant Green Bile 2%, as well as with additional biochemical tests.

The most probable number of organisms in 100 mL of the original water sample can be calculated using the following table.⁷

QUANTITY OF WATER IN EACH TUBE	50 mL 10 mL		MOST PROBABLE NUMBER (MPN) OF COLIFORMS IN 100 mL IN SAMPLE
	1	2	
NUMBER OF TUBES USED	0	0	0
Number of Tubes	0	1	1
Giving Positive	0	2	2
Reaction	0	3	4
	0	4	5
	0	5	7
	1	0	2
	1	1	3
	1	2	6
	1	3	9
	1	4	16
	1	5	+18

Limitations of the Procedure

1. The performance of the medium is significantly affected by pH. Avoid overheating the broth. Check the pH of each lot before proceeding with testing.
2. Due to the nutritional requirements of the organisms, some organisms other than coliform bacteria may grow in the medium with production of acid and gas. Test all presumptive-positive tubes to confirm the presence of *Escherichia coli*.

References

1. Gray. 1959. J. Hyg., Camb. 57:249.
2. Gray. 1964. J. Hyg., Camb. 62:495.
3. P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies. 1968. J. Hyg., Camb. 65:67.
4. Joint Committee of the P. H. L. S. and the Standing Committee of Analysts. 1980. J. Hyg., Camb. 85:35.
5. Abbiss, Wilson, Blood and Jarvis. 1981. J. Appl. Bact. 51:121.
6. Holbrook, Anderson and Baird-Parker. 1980. Food Technol. Aust. 32:78.
7. Departments of the Environment, Health & Social Security, and P.H.L.S. 1982. The bacteriological examination of drinking water supplies. Report on Public Health and Medical Subjects No. 71., H.M.S.O., London, England.

Availability

Difco™ Minerals Modified Glutamate Broth

Cat. No. 218501 Dehydrated – 500 g