

- Alternatively, plates of Lactobacilli MRS Agar can be used for direct recovery of organisms using the streak inoculation technique.
- Incubate agar plates at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide.

Broth Enrichment

- Samples can be inoculated directly into Lactobacilli MRS Broth.
- Incubate broth tubes at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere.
- Subculture growth in broth tubes to appropriate solid media.

Expected Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar or as turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.^{2,3}

Limitation of the Procedure

Organisms other than lactobacilli may grow in these media. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

References

- deMan, Rogosa and Sharpe. 1960. J. Appl. Bacteriol. 23:130.
- Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Downes and Ito(ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Lactobacilli MRS Agar

COMPF SMD
 Cat. No. 288210 Dehydrated – 500 g*

Difco™ Lactobacilli MRS Broth

COMPF SMD
 Cat. No. 288130 Dehydrated – 500 g*
 288110 Dehydrated – 2 kg*
 288120 Dehydrated – 10 kg*

*Store at 2-8°C.

Lactose Broth

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Lactose Broth is used for detection of the presence of coliform organisms, as a pre-enrichment broth for salmonellae and in the study of lactose fermentation of bacteria in general.

Summary and Explanation

Lactose Broth was formulated in accordance with recommendations of the American Public Health Association (APHA) and the American Water Works Association for testing dairy products and water for the presence of coliform organisms.^{1,2} This medium was, but no longer is, listed as an alternative to Lauryl Sulfate Broth in the presumptive portion of the Standard Total Coliform Multiple-Tube (MPN) Test for water analysis provided that it had been demonstrated not to increase the frequency of false-positives nor mask coliforms.³ It is one of the recommended media in the *Compendium of Methods for the Microbiological Examination of Foods* for pre-enrichment when *Salmonella* organisms are suspected in foods.⁴ It is included in the *USP* for use in the performance of Microbial Limit Tests for *Salmonella* species and *Escherichia coli*.⁵

Principles of the Procedure

The peptone and beef extract provide essential nutrients for bacterial metabolism. Lactose provides a source of fermentable carbohydrate for coliform organisms. Growth with the formation of gas is a presumptive test for coliforms.



Formulae

Difco™ Lactose Broth

Approximate Formula* Per Liter

Beef Extract	3.0	g
Peptone	5.0	g
Lactose	5.0	g

BBL™ Lactose Broth

Approximate Formula* Per Liter

Beef Extract	3.0	g
Pancreatic Digest of Gelatin	5.0	g
Lactose	5.0	g

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



User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Lactose Broth

Dehydrated Appearance:	Light beige to light tan, free flowing, homogeneous.
Solution:	1.3% solution, soluble in purified water upon slight warming. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 1.3% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Lactose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. After incubation, add 1-2 drops of 1% phenol red solution to observe acid production.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
<i>Enterobacter aerogenes</i>	13048	30-300	Good	+	+
<i>Escherichia coli</i>	25922	30-300	Good	+	+
<i>Enterococcus faecalis</i>	19433	30-300	Good	+	-
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	6539	30-300	Good	-	-

Identity Specifications

BBL™ Lactose Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.3% solution, soluble in purified water. Solution is pale to light, tan to yellow, clear to slightly hazy.
Prepared Appearance:	Pale to light, tan to yellow, clear to slightly hazy.
Reaction of 1.3% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ Lactose Broth

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
<i>Enterobacter aerogenes</i>	13048	10 ³ -10 ⁴	Good	+	+
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Good	+	-
<i>Escherichia coli</i>	11775	10 ³ -10 ⁴	Good	+	+
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	+	+
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	13311	10 ³ -10 ⁴	Good	-	-

Directions for Preparation from Dehydrated Product

- Dissolve 13 g of the powder in 1 L of purified water.
- Dispense in test tubes containing inverted Durham tubes, in 10 mL amounts for testing samples of 1 mL or less. For testing 10 mL quantities of samples, dissolve 26 g of the powder in 1 L of purified water and distribute in 10 mL amounts. The concentration of the medium should be varied according to the size of the test samples. The concentration of broth plus sample should approximate 1× for proper performance. (In broth concentrations higher than 2×, a reduction in clarity may be noticed.)
- Autoclave at 121°C not exceeding 15 minutes.
- After autoclaving, cool the broth as quickly as possible.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to the official test procedures for the detection of coliforms in the compendia of methods for the microbiological examination of foods and pharmaceutical articles.^{4,5}

Expected Results

After incubation at 35 ± 2°C for 24 ± 2 hours, examine tubes for turbidity and for gas production in the Durham tubes. If no gas has formed and been trapped in the inverted tube, reincubate and reexamine after 48 ± 3 hours.

Turbidity of the medium accompanied by formation of gas in any amount in the Durham tubes within 48 ± 3 hours is a positive presumptive test for the presence of coliforms in the sample. The result should be confirmed by additional standard testing.

References

- American Public Health Association. 1946. Standard methods for the examination of water and sewage, 9th ed. APHA, New York, N.Y.
- American Public Health Association. 1948. Standard methods for the examination of dairy products, 9th ed. APHA, New York, N.Y.
- Rand, Greenberg and Taras (ed.). 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
- Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
- United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Lactose Broth

	AOAC	BAM	COMP	EP	EPA	SMD	USP
Cat. No.	243000						
	211835						
	241000						
	242000						
	290701						

BBL™ Lactose Broth

	AOAC	BAM	COMP	EP	EPA	SMD	USP
Cat. No.	211333						
	211334						
	212255						

*Store at 2-8°C.