

Directions for Preparation from Dehydrated Product

Difco™ Lauryl Tryptose Broth

1. Suspend 35.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Dispense required amounts into tubes containing inverted fermentation vials (see table).
4. Autoclave at 121°C for 15 minutes. Cool the broth as quickly as possible.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Lauryl Sulfate Broth

1. Suspend 35.6 g of the powder in 1 L of purified water.
2. 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 71.2 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 (see table).
3. Autoclave at 121°C for 15 minutes. After autoclaving, cool the broth as quickly as possible.
4. Test samples of the finished product for performance using stable, typical control cultures.

Preparation of Lauryl Tryptose (Sulfate) Broth⁴

INOCULUM mL	AMOUNT OF MEDIUM IN THE TUBE mL	VOLUME OF MEDIUM+INOCULUM mL	DEHYDRATED MEDIUM REQUIRED g/L
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

NOTE: Refrigerated broth generally becomes cloudy or forms precipitates but clears upon warming to room temperature.

However, clarity is not important because only gas production is significant.

Procedure

Refer to the official test procedures for the detection of coliforms in the compendia of methods for microbiological examination of foods, dairy products and waters.²⁻⁵

Expected Results

After incubation of the tubes with loosened caps at 35 ± 0.5°C for 24 hours, examine for turbidity and for gas production in the Durham fermentation tubes. If no gas has formed and been trapped in the inverted tube, reincubate and reexamine after 48 hours.²⁻⁵

Turbidity of the medium accompanied by formation of gas in any amount in the Durham tubes within 48 hours is a positive presumptive test for the presence of coliforms in the sample.²⁻⁵ The result should be confirmed by additional standard testing.

References

1. Mallmann and Darby. 1941. Am. J. Public Health 31:127.
2. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
3. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2000. Official methods of analysis of AOAC International. 17th ed. AOAC International. Gaithersburg, Md.

Availability

Difco™ Lauryl Tryptose Broth

	AOAC	BAM	CCAM	COMPF	EPA	ISO	SMD	SMWW	USDA
Cat. No. 224140									
224150									
224120									
224130									

BBL™ Lauryl Sulfate Broth

	AOAC	BAM	CCAM	COMPF	EPA	ISO	SMD	SMWW	USDA
Cat. No. 211338									
211339									
294369									

Lauryl Tryptose Broth with MUG Lauryl Sulfate Broth with MUG

Intended Use

Lauryl Tryptose Broth with MUG and Lauryl Sulfate Broth with MUG, which are also known as Lauryl Sulfate Tryptose Broth with MUG (LST-MUG), are used for the detection of *Escherichia coli* in water, food and dairy samples by a fluorogenic procedure.

Summary and Explanation

E. coli is used as an indicator organism of unsanitary conditions. A number of selective media are recommended for use in enrichment, presumptive identification and confirmatory procedures for demonstrating the presence of coliforms in

material of sanitary importance. These procedures require the incubation of test media for up to 7 days.

The presence of the fluorogenic compound, MUG (4-methylumbelliferyl-β-D-glucuronide), in this medium permits the rapid detection of *E. coli* when the medium is observed for fluorescence using a long-wave UV light source, and further confirmation is not required.^{1,2} MUG detects anaerogenic strains which may not be detected in the conventional procedure.¹

Feng and Hartman, using a MUG-containing medium in microtitration plates, reported β-glucuronidase activity in 96% of *E. coli*, 100% of enterotoxigenic *E. coli*, 17% of *Salmonella*

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™ Lauryl Tryptose Broth with MUG

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.57% solution, soluble in purified water upon warming. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.57% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Lauryl Tryptose Broth with MUG

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 ± 2 hours or longer, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS	FLUORESCENCE
<i>Enterobacter aerogenes</i>	13048	30-100	Good	+	–
<i>Escherichia coli</i>	25922	30-100	Good	+	+
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	30-100	Good	–	–
<i>Staphylococcus aureus</i>	25923	3 × 10 ² -10 ³	Marked to complete inhibition	–	–

*Gas production positive within 48 ± 3 hours.

Identity Specifications

BBL™ Lauryl Sulfate Broth with MUG

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.57% 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 3.57% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

BBL™ Lauryl Sulfate Broth with MUG

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	GAS	FLUORESCENCE
<i>Enterobacter aerogenes</i>	13048	10 ³ -10 ⁴	Good	+	–
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Partial to complete inhibition	–	–
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	+	+

spp., and 40% of *Shigella* spp., while all other genera tested were negative; most reactions occurred within 4 hours, but some weakly β-glucuronidase-positive strains required overnight incubation.¹ In the presence of large numbers of *Proteus vulgaris*, which may suppress gas production by *E. coli*, fluorescence due to *E. coli* was detected within 15 hours.¹

In a comparison, with conventional methods, Robison reported 94.8% agreement, a false-positive rate of 4.8%, attributable to the presence of streptococci in the samples, and no false-negatives.²

These media are included in the compendia of methods for the detection and enumeration of coliform organisms in food³ and dairy⁴ products and in the *Official Methods of Analysis of AOAC International*.⁵

Principles of the Procedure

Lactose is a source of energy for organisms. Peptone provides additional nutrients. The phosphate compounds provide buffering capacity. Sodium lauryl sulfate is inhibitory to many organisms but not for coliforms.

The substrate 4-methylumbelliferyl-β-D-glucuronide is hydrolyzed by an enzyme, β-glucuronidase, possessed by most *E. coli* and a few strains of *Salmonella*, *Shigella* and *Yersinia*,

to yield a fluorescent end product, 4-methylumbelliferone.^{1,2} Development of fluorescence permits the detection of *E. coli* in pure or mixed cultures within 4-24 hours following inoculation and incubation of the medium.

Formulae

Difco™ Lauryl Tryptose Broth with MUG

Approximate Formula* Per Liter	
Tryptose	20.0 g
Lactose	5.0 g
Dipotassium Phosphate	2.75 g
Monopotassium Phosphate	2.75 g
Sodium Chloride	5.0 g
Sodium Lauryl Sulfate	0.1 g
MUG (4-methylumbelliferyl-β-D-glucuronide)	0.05 g

BBL™ Lauryl Sulfate Broth with MUG

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	20.0 g
Lactose	5.0 g
Dipotassium Phosphate	2.75 g
Monopotassium Phosphate	2.75 g
Sodium Chloride	5.0 g
Sodium Lauryl Sulfate	0.1 g
4-Methylumbelliferyl-β-D-glucuronide	0.05 g

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

Directions for Preparation from Dehydrated Product

Difco™ Lauryl Tryptose Broth with MUG

1. Suspend 35.7 g of the powder in 1 L of purified water and warm slightly to dissolve completely.
2. Dispense into test tubes containing inverted fermentation vials.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Lauryl Sulfate Broth with MUG

1. Dissolve 35.7 g of the powder in 1 L of purified water.
2. 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 71.4 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.
3. Autoclave at 121°C, preferably for 12 minutes, but not exceeding 15 minutes. After autoclaving, cool the broth as quickly as possible.
4. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Refrigerated broth generally becomes cloudy or forms precipitates but clears upon warming to room temperature. However, clarity is not important because only gas production is significant.

Procedure

Follow standard methods for the test being performed.³⁻⁵ Observe the medium periodically during the incubation period for the development of fluorescence, using a long-wave UV light source (approximately 366 nm) as well as for characteristic growth and/or gas production.

Expected Results

The presence of *E. coli* is detected by the appearance of fluorescence throughout the tube.

References

1. Feng and Hartman. 1982. Appl. Environ. Microbiol. 43:1320.
2. Robison. 1984. Appl. Environ. Microbiol. 48:285.
3. Downes and Ito. 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. Marshall (ed.) 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.

Availability

Difco™ Lauryl Tryptose Broth with MUG

AOAC BAM COMPF SMD SMWW
 Cat. No. 211740 Dehydrated –100 g
 211744 Dehydrated – 500 g

BBL™ Lauryl Sulfate Broth with MUG

AOAC BAM COMPF SMD SMWW
 Cat. No. 298076 Dehydrated –500 g

Lecithin Lactose Agar

Intended Use

Lecithin Lactose Agar is recommended for the isolation and differentiation of histotoxic clostridia from clinical specimens.¹ It is particularly useful in differentiating *Clostridium perfringens*, *C. sordellii*, *C. novyi*, *C. septicum* and *C. histolyticum*.

Summary and Explanation

Culture media containing egg yolks were useful in isolating, cultivating and identifying species of histotoxic clostridia. In 1948, McClung and Toabe reported on the use of egg yolk medium for this purpose.¹ Willis and Hobbs added milk and lactose to the egg yolk in a formulation designed to group clostridia on the basis of production of lecithinase, hydrolysis of casein and lactose fermentation.² Neomycin sulfate was included in their formulation in order to make it a selective medium.

Willis, in response to problems in the obtaining and processing of antibiotic-free eggs, developed an egg-free medium in which purified lecithin was substituted for the egg yolk.³ In order to further refine the growth-promoting and selective properties, Ellner and O'Donnell developed the formulation, designated as Lecithin Lactose Agar, in which a decreased concentration of neomycin was employed and to which sodium azide was

added.⁴ This medium continues to be an important one for visualizing and characterizing the histotoxic clostridia.

Principles of the Procedure

Lecithin Lactose Agar provides differentiation of clostridia by the demonstration of lecithinase production and lactose fermentation. The medium is rendered selective by the addition of neomycin and sodium azide. Gram-negative and aerobic gram-positive rods are inhibited. Growth of gram-positive cocci is suppressed.

On this medium, the production of a zone of opalescence surrounding colonies indicates lecithinase production. A yellow color surrounding colonies indicates lactose fermentation due to the effect of the lowered pH on the bromcresol purple indicator.

Procedure

As soon as possible after receipt in the laboratory, inoculate the specimen onto a reduced Lecithin Lactose Agar plate and streak for isolation. Since some anaerobes may be inhibited due to the selective nature of the medium, it is advisable to include a nonselective medium such as CDC Anaerobe 5% Sheep Blood Agar.

