m Endo Agar LES

Intended Use

m Endo Agar LES is used for enumerating coliforms in water by membrane filtration.

Summary and Explanation

McCarthy, Delaney and Grasso[1] formulated Endo Agar LES (Lawrence Experimental Station) for testing water for coliform bacteria by a two-step membrane filter procedure using Lauryl Tryptose Broth as a preliminary enrichment. They recovered higher numbers of coliforms by this method compared with the one step technique using m Endo Broth.

The American Public Health Association specifies using m Endo Agar LES in the standard total coliform membrane filtration procedure for testing drinking water[2] and bottled water.[3] It is also specified for use in the completed phase of the standard total coliform fermentation technique.[2] The coliform bacteria are bacteria that produce a red colony with a metallic (golden) sheen within 24 hours incubation at 35°C on an Endo-type medium.

Principles of the Procedure

m Endo Agar LES contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium chloride maintains the osmotic balance of the medium. Sodium desoxycholate and sodium lauryl sulfate are added as inhibitors. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution. Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose-nonfermenting bacteria form clear, colorless colonies.

Formula

Difco™ m Endo Agar LES

Approximate Formula* Per Liter		
Yeast Extract	1.2	g
Casitone	3.7	g
Thiopeptone	3.7	g
Tryptose	7.5	g
Lactose	9.4	g
Dipotassium Phosphate	3.3	g
Monopotassium Phosphate	1.0	g
Sodium Chloride	3.7	g
Sodium Desoxycholate	0.1	g
Sodium Lauryl Sulfate	0.05	g
Sodium Sulfite	1.6	g
Basic Fuchsin	0.8	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 51 g of the powder in 1 L of purified water containing 20 mL of 95% ethanol. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Place a membrane filter absorbent pad inside the cover of a Petri dish.
2. Add 1.8-2.0 mL Lauryl Tryptose Broth or Lauryl Sulfate Broth to each pad.
3. Run the water sample through a membrane filter.
4. Place the filter, top side up, onto the pad containing Lauryl Tryptose Broth or Lauryl Sulfate Broth. Use a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35 ± 0.5°C for 1.5-2.5 hours. Transfer the membrane from the pad to the surface of the m Endo Agar LES medium in the Petri dish bottom, keeping the side on which the bacteria have been collected facing upward.
6. Leave the filter pad in the lid and incubate the plates in the inverted position at 35 ± 0.5°C for 22 ± 2 hours.
7. Observe and count all colonies that are red and have a metallic sheen.

Expected Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

User Quality Control

Identity Specifications

Difco™ m Endo Agar LES

Dehydrated Appearance:	Purple, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water containing 2% ethanol upon boiling. Solution is pinkish-red, slightly opalescent to opalescent with precipitate.
Prepared Appearance:	Rose colored, slightly opalescent, with precipitate.
Reaction of 5.1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ m Endo Agar LES

Prepare the medium per label directions. Use the membrane filter technique to inoculate filters and preincubate on pads saturated with Lauryl Tryptose Broth or Lauryl Sulfate Broth at 35 ± 0.5°C for 1.5-2 hours. Transfer filters to plates of m Endo Agar LES and incubate at 35 ± 0.5°C for 22 ± 2 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	25922	30-80	Good	Red with sheen
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-80	Good	Pink
Staphylococcus aureus	25923	10^3	Marked to complete inhibition	–

References

1. McCarthy, Delaney and Grasso. 1961. Water Sewage Works *108*:238.
2. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
3. Kim and Feng. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ m Endo Agar LES

COMPF SMD SMWW

Cat. No.	273610	Dehydrated – 100 g
	273620	Dehydrated – 500 g