m ENDO AGAR

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.

PRINCIPLE

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.

REAGENTS (FORMULA)

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
Deionized Water	1000.0	ml

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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 0.5°C for 22± 2 hours	Growth, Red with sheen
Staphylococcus aureus ATCC 25923	35 ± 0.5°C for 22± 2 hours	Complete Inhibition

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BIBLIOGRAPHY

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.

CBSA life

155-196 Innovation Drive, Winnipeg, MB, R3T 2N2, Canada

Phone: +1 (204) 269-2255 Email: info@cbsalife.com Website: https://cbsalife.com