EOSIN METHYLENE BLUE (EMB) MEDIA

PRODUCTS:

Plated Media:

Eosin Methylene Blue Agar, Levine	P1600
Eosin Methylene Blue Agar, Modified	P3430
Eosin Methylene Blue Agar with Tetracycline	P1605

PURPOSE:

EMB Agar is selective media used for the isolation and differentiation of enteric bacilli, especially coliforms, in clinical specimens, water, and dairy products. It is also used to differentiate *Candida albicans* for other yeast species.

PRINCIPLE:

Holt, Harris, Teague[2] first developed Eosin Methylene Blue agar. The eosin dye inhibits growth of gram-postive bacteria and combines with the methylene blue indicator to produce a color change whenever lactose or sucrose are fermented. The modified formulation of Holt, Harris and Teague further balances eosin and methylene blue to optimize differentiation between organisms which ferment these carbohydrates and those that do not. This medium does not allow discrimination between which carbohydrate is fermented. *Yersinia enterocolitica*, which ferments sucrose, but not lactose, will produce the same purple-black colony as lactose-fermenting bacteria.

The Levine[5] formula eliminates the sucrose and doubles the lactose concentration. As in the HHT-modified formulation, lactose fermenters appear as colonies with blue-black centers and non-lactose fermenters appear as clear to opaque colonies. Because the Levine formulation contains lactose as the only fermentable carbohydrate, reactions are more comparable with MacConkey Agar.

Candida albicans can also be differentiated using the Levine formula from other *Candida* and *Cryptococcus* species by its ability to produce germ tubes within 3 hours, and pseudohyphae and budding cells at 18-24 hours when incubated at 35°C in 5-10% CO_2. The addition of tetracycline to the Levine formulation aids in the selection of *C.albicans* from clinical sources that are contaminated with bacteria.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) EMB Agar, Levine:

Pancreatic Hydrolysate of Gelatin	10.0 g
Lactose	10.0
Dipotassium Phosphate	2.0
Agar	15.0
Eosin Y	0.4
Methylene Blue	65.0 mg

Final pH 7.1 ± 0.2 at 25°C

(2) EMB Agar, Modified:

Same as **(1)** except it contains 5.0 g of Lactose and also 5.0 g of Sucrose.

Final pH 7.1 ± 0.2 at 25°C

(3) EMB Agar, (Levine) with Tetracycline:

Same as **(1)** with 0.1g of Tetracycline

PRECAUTIONS: *

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. The photosensitive dyes in the medium may inhibit growth of certain bacteria, mainly *Proteus sp*., if exposed to light[1]. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: This medium is only moderately inhibitory; some staphylococci and streptococci will grow, appearing as small, pinpoint

colonies. EMB will support the growth of all *Enterobacteriaceae* including *Salmonella* and *Shigella*. Because there is no inhibitory effect in non-pathogenic enteric bacteria, EMB is not recommended for isolation of stool specimens for enteric pathogens.

The metallic sheen observed on the strongly fermenting *E.coli* can be helpful in identifying that organism; however, other species of bacteria may produce a sheen (e.g., *Yersinia enterocolitica*) and not all strains of *E.coli* will exhibit this sheen. Further testing may be required to establish complete identification of the organism. Biochemical and/or serological procedures are found in standard reference texts.[3,5,6]

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extreme heat and cold and transported to the laboratory without delay.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate the specimen onto the media surface using standard microbiological procedures to obtain isolated colonies. Incubate aerobically at 35°C for 18-24 hours; for *Candida albicans* identification, incubate at 35°C in 5-10% CO_2 for 3-24 hours.

Interpretation:

Escherichia coli	Blue-black, dark centered colony with green, metallic sheen
Salmonella species	Colorless or transparent, light-purple colonies
Klebsiella species	Mucoid brownish colony with blue-black center
Proteus species	Smooth, translucent, colorless colonies
Enterococcus faecalis	Small, pin-point, clear colonies
Candida albicans (in CO_2)	At 2-4 hours incubation, germ tubes can be observed when plate is examined under low magnification using a microscope At 24 hours the characteristically feathery colonies will demonstrate pseudohyphae and budding cells under low magnification
Candida species	No germ tube production. Colonies will appear as smooth, circular, and cream colored

For the definitive identification of *Enterobacteriaceae*, additional biochemical test must be performed.

Materials Required but Not Provided: Standard microbiological supplies and equipment are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	Expected Results:
Salmonella typhimurium (14028)	Growth
Escherichia coli (25922)	Growth
Enterococcus faecalis (29212)	Growth
Proteus mirabilis (12453)	Growth
Candida albicans (10231)	Growth

Key: See "Interpretation"

User Quality Control: Check for signs of contamination and deterioration. Eosin Methylene Blue media should appear translucent and wine (reddish-purple) in color.

BIBLIOGRAPHY:

1. Girolami, R.L., and J.M. Stamm. *Appl. Environ. Mircobiol.*, 31:141, 1976.
2. Holt-Harris, J.E., and O. Teague, *J. Infect. Dis.*, 18:596,1916.
3. Koneman, E. W., et.al., *Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed., J.B. Lippincott, Philadelphia, 2005.
4. Murray, P.R., et. al., *Manual of Clinical Microbiology*, 7th ed. American Society for Microbiology, Washington D.C. 1999.
5. Levine, M. J. Infect. Dis. 23:43, 1968.
6. MacFaddin, J.F., *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, vol. 1, Williams and Wilkins Baltimore, 1985.

*For more detailed information, consult appropriate references.

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