EMB (HHT) AGAR

INTENDED USE
Remel EMB (HHT) Agar is a solid medium recommended for use in qualitative procedures for isolation and differentiation of gram-negative enteric bacilli from clinical specimens.

SUMMARY AND EXPLANATION
Eosin Methylene Blue Agar (EMB Agar) was originally developed by Holt-Harris and Teague.[1] The combination of dyes in this medium and the incorporation of lactose and sucrose provide a differential plating medium which facilitates the identification of fermentative, gram-negative bacilli.

PRINCIPLE
Gelatin peptone supplies amino acids and essential nutrients necessary for the growth of bacteria. Eosin dye combines with methylene-blue indicator to produce a color change when lactose or sucrose is fermented. Lactose-fermenting coliforms, such as *Escherichia coli*, produce blue-black colonies with a green metallic sheen due to the amide bonding of the dyes in an acid condition. Other coliforms form mucoid, pink-brown colonies in a less acidic condition. Lactose nonfermenters, such as *Shigella* and *Salmonella*, form transparent, colorless, or amber colonies which are easily distinguished from coliforms. Eosin and methylene-blue dyes are also selective agents which inhibit the growth of gram-positive organisms.

REAGENTS (CLASSICAL FORMULA)*

Gelatin Peptone	10.0	g	Eosin Y	0.4	g
Lactose	5.0	g	Methylene Blue	65.0	mg
Sucrose	5.0	g	Agar	13.5	g
Dipotassium Phosphate	2.0	g	Demineralized Water	1000.0	ml

pH 7.2 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is received on a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plate protected from light in an aerobic atmosphere at 33-37°C for 18-24 hours.
4. Examine plate for typical colony morphology and the formation of a green metallic sheen.

QUALITY CONTROL
All lot numbers of EMB (HHT) Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[2] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Escherichia coli* ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Blue-black colonies w/ green metallic sheen
Proteus mirabilis ATCC® 12453	Aerobic, 18-24 h @ 33-37°C	Clear colonies, swarming
**Salmonella enterica* serovar Typhimurium ATCC® 14028	Aerobic, 18-24 h @ 33-37°C	Clear colonies
**Enterococcus faecalis* ATCC® 29212	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, 18-24 h @ 33-37°C	Inhibition (partial to complete)

*CLSI recommended organism

LIMITATIONS
1. EMB (HHT) Agar contains a photosensitive dye which may inhibit growth of certain bacteria, mainly *Proteus* spp. when stored in the light. Store and incubate media protected from light.[3]
2. *E. coli* does not always produce a green metallic sheen on EMB (HHT) Agar, and the presence of a green metallic sheen does not presumptively identify *E. coli*.[4]
3. Some strains of *Salmonella* and *Shigella* will not grow on EMB (HHT) Agar.[3]

BIBLIOGRAPHY

1. Holt-Harris, J.E. and O. Teague. 1916. J. Infect. Dis. 18:596.
2. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
4. Girolami, R.L. and J.M. Stamm. 1976. Appl. Environ. Microbiol. 31:141.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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