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TECHNICAL PRODUCT INFORMATION

EMB AGAR [Levine]
Catalog No. P1301 - Mono Plate

BA/EMB Bi-plate
Catalog No. P3055

EMB/MAC Bi-plate
Catalog No. P3060

INTENDED USE:
Eosin Methylene Blue Agar [EMB] is a differential plating medium recommended for use in the isolation of Gram negative enteric bacilli.

HISTORY/SUMMARY:
Holt, Harris, and Teaque first developed Eosin Methylene Blue Agar. By combining the eosin and methylene blue indicator system in a medium containing lactose and sucrose, these investigators found a medium which would readily distinguish members of the *Escherichia* and *Enterobacter* species from other Gram negative bacilli.

EMB will satisfactorily support the growth of enteric pathogens *Salmonella* and *Shigella*. Due to the lack of selective agents, however, the medium has no inhibitory effect on the non-pathogenic enteric bacilli. For this reason, EMB is not recommended for selective isolation in examining fecal specimens for enteric pathogens. The exception would be the examination of specimens for serotypes of *E.coli* associated with infantile diarrhea.

The Levine formula does not contain sucrose and the lactose concentration is doubled. The American Public Health Association [APHA] recommends this medium for use in diagnostic procedures and the examination of water and dairy products. The APHA cautions that although the Holt-Harris and Teaque medium [containing both lactose and sucrose] may be used for the detection of intestinal pathogens, it should not be used in water bacteriology. In the examination of water, the Levine medium is used in the confirmed and completed tests; and in the completed test for coliforms in dairy products.

The Levine medium has also found application in mycological studies. Weld proposed its use with added chlortetracycline for the rapid selection of *Candida* species from clinical sources.

PRINCIPLES:
The balance of eosin Y and methylene blue is the formula allows for the differentiation of lactose fermenters from non-lactose fermenters. *Escherichia coli* forms blue-black colonies with a distinctive metallic sheen, the result of an amide bonding of the eosin and methylene blue in the strongly acidic condition. Other coliforms form mucoid, brownish colonies in a much less acidic condition. *Salmonella* and *Shigella* form transparent, colorless to amber colonies readily distinguishable from coliforms.

The Holt-Harris and Teaque formula contains both lactose and sucrose. Without sucrose, the slow lactose fermenters growing on this medium would mimic the appearance of enteric pathogens. As most slow lactose fermenters readily ferment sucrose, the inclusion of sucrose in the formula reduces the incidence of "false positive" interpretations.
The eosin dye has a capability of inhibiting gram-positive bacteria.

FORMULA:

EOSIN METHYLENE BLUE AGAR [LEVINE]

Component (per liter of purified water)	Amount
Pancreatic Digest of Gelatin	10.0 g
Lactose	10.0 g
Potassium Phosphate, Dibasic	2.0 g
Eosin Y	0.4 g
Methylene Blue	0.065 g
Agar	15.0 g

Final pH: 7.1 ± 0.2 @ 25°C

PRECAUTIONS:
Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director.
This product is for In Vitro Diagnostic Use.

STORAGE:
Store at 2 to 8°C, do not use if discolored, dehydrated or obviously contaminated. Use media prior to expiration date. Autoclave all inoculated plates prior to discarding.

SPECIMEN COLLECTION:
Freshly passed stool specimens collected early in the disease prior to institution of an antimicrobic regimen are recommended for recovery of enteric pathogens. Rectal swabs should be collected from persons with diarrhea disease but cannot be relied upon to yield maximal results. When properly collected specimens cannot be cultured soon after collection, they should be preserved in a transport solution such as Stuarts, Amies, or Cary-Blair. Stool specimens preserved in transport media, fresh feces, rectal swab, and feces from soiled diapers should be suspended in a tube of GN Broth on a cotton-tipped swab supported by a cotton plug.

PROCEDURE:
Direct plating:

1. Emulsify feces sample in saline if necessary.
2. Shake off loop before streaking.
3. Use moderate inoculum to streak EMB agar plates.
4. If swabs are used:
    a. Roll swabs in a corner of the plate and streak for isolation from this area.
    b. Incubate streaked plates and inoculate enrichment broth 18-24 hours at 35°C.
5. If GN broth is used:
    a. Check for growth at 6 hours.
    b. When growth is apparent, streak to EMB Agar.

PERFORMANCE TEST:
Approval for manufacture of each lot is based on the demonstrated effectiveness in cultivating and/or differentiating the micro-organisms listed below. The growth characteristics and reactions listed are typical observations made on challenged samples:

TEST ORGANISM	RESULTS
ATCC# 25922 *Escherichia coli*	Black with metallic sheen
ATCC# 14028 *Salmonella typhimurium*	Transparent to colorless
ATCC# 29212 *Enterococcus faecalis*	Inhibited

Other typical organism reactions:	
Staphylococcus aureus	Inhibited
Candida albicans	White, dry
Enterobacter aerogenes	Pink colonies with black centers
Shigella flexneri	Transparent

QUALITY CONTROL:
The performance characteristics as noted above may be confirmed by the laboratory through a judicious selection of organisms such as those recorded above.

REFERENCES:

1. Journal of Infectious Disease, 18:596, 1916
2. American Journal of Clinical Pathology 35:476, 1965
3. Technical Bulletin Regs. Medical Technology, 37:222, 1967
4. Difco & BBL Manual, 2003 Pages 218-220