



QUALITY CONTROL PROCEDURES

I INTRODUCTION

EMB Agar, Modified (formula of Holt-Harris and Teague) is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric bacilli from both clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

- 1. Inoculate representative samples with dilutions of the cultures listed below.
a. Using an 18-24 h broth culture of Enterococcus faecalis diluted to yield 10^4-10^5 CFU/plate, spread inoculate using a sterile glass spreader. For the remaining organisms, use an 18-24 h broth culture diluted to yield 10^3-10^4 CFU/plate. Streak inoculate E. coli. Spread inoculate remaining organisms.
b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
c. Include Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18-24 h for growth, colony size, pigmentation and selectivity.
3. Expected Results

Table with 3 columns: CLSI Organisms, ATCC™, Recovery. Rows include Escherichia coli, Salmonella choleraesuis, Enterococcus faecalis, and Shigella flexneri.

\*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- 1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

EMB Agar, Modified is a differential and selective plating medium for the isolation of gram-negative enteric bacilli.

V SUMMARY AND EXPLANATION

In 1904, Endo developed a culture medium for the isolation of typhoid bacilli from feces, and this medium was widely used in the years immediately following its development. According to Holt-Harris and Teague, the chief disadvantage of the Endo medium was that the red color of the coliform colonies diffused through the surrounding medium.

The original EMB Agar formulation of Holt-Harris and Teague was modified by Levine who described his medium in a 1918 publication. Over the years, it is the Levine Eosin Methylene Blue formulation which has achieved dominant status.

VI PRINCIPLES OF THE PROCEDURE

EMB Agar, Modified contains eosin Y and methylene blue dyes which inhibit gram-positive bacteria to a limited degree. The dyes also serve as differential indicators in response to the fermentation of lactose and/or sucrose by microorganisms.

Coliforms produce blue-black colonies whereas Salmonella and Shigella colonies are colorless or have a transparent amber color. Escherichia coli colonies may show a characteristic green metallic sheen due to the rapid fermentation of lactose.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of nonpathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

VII REAGENTS

EMB Agar, Modified, Holt-Harris and Teague

Approximate Formula\* Per Liter Purified Water

Table listing reagents and quantities: Pancreatic Digest of Gelatin (10.0 g), Lactose (5.0 g), Sucrose (5.0 g), Dipotassium Phosphate (2.0 g), Agar (13.5 g), Eosin Y (0.4 g), Methylene Blue (0.065 g).

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

**Warnings and Precautions:** For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>4-7</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

**VIII SPECIMEN COLLECTION AND HANDLING**

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>8,9</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

**IX PROCEDURE**

**Material Provided:** EMB Agar, Modified

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2°C for 18–24 h in an aerobic atmosphere.

**User Quality Control:** See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

**X RESULTS**

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on EMB Agar, Modified, is as follows:

- E. coli* .....Large, blue-black, green metallic sheen
- Enterobacter/Klebsiella* .....Large, mucoid, blue-black
- Proteus* .....Large, colorless
- Salmonella* .....Large, colorless to amber
- Shigella* .....Large, colorless to amber
- Pseudomonas* .....Irregular, colorless
- Gram-positive bacteria .....No growth to slight growth

**XI LIMITATIONS OF THE PROCEDURE**

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>8-13</sup>

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

**XII AVAILABILITY**

Cat. No.	Description
221354	BBL™ EMB Agar, Modified, Holt-Harris and Teague, Pkg. of 20 plates
221355	BBL™ EMB Agar, Modified, Holt-Harris and Teague, Ctn. of 100 plates

**XIII REFERENCES**

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