EOSIN METHYLENE BLUE AGAR

INTENDED USE

Eosin Methylene Blue Agar is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric bacilli from both clinical and nonclinical specimens.

SUMMARY AND EXPLANATION

In 1904, Endo developed a culture medium for the isolation of typhoid bacilli from feces,[1] and this medium was widely used in the years immediately following its development. According to Holt-Harris and Teague,[2] the chief disadvantage of the Endo medium was that the red color of the coliform colonies diffused through the surrounding medium. When larger numbers of these colonies were present on the agar surface, the colorless colonies of the typhoid organisms and other lactose nonfermenters were masked and often overlooked. In 1916, these two scientists reported on the development of a new medium in which the dyes, eosin Y and methylene blue, were incorporated. Differentiation between lactose fermenters and lactose nonfermenters on this formulation was greatly improved since color diffusion into the agar was eliminated.

The original EMB Agar formulation of Holt-Harris and Teague was modified by Levine who described his medium in a 1918 publication.[3] Levine simplified the original formula by using a single peptone as a base and supplementing it with dipotassium phosphate as a buffer and by deleting the sucrose and increasing the concentration of lactose. The concentration of methylene blue was later reduced because of increased purity of the dye. This provided the current ratio of eosin to methylene blue of approximately 6:1. Over the years, it is the Levine Eosin Methylene Blue formulation that has achieved dominant status.

PRINCIPLE

Eosin Methylene Blue Agar contains eosin Y and methylene blue dyes that 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 due to the taking up of an eosin-methylene blue dye complex by the bacterial cells when the pH drops. *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 non-pathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic bacterial strains by additional biochemical tests.

REAGENTS (FORMULA)

Pancreatic Digest of Gelatin	10.0	g
Lactose	10.0	g
Dipotassium Phosphate	2.0	g
Eosin Y	0.4	g
Methylene Blue	65.0	mg
Agar	13.5	g
Deionized Water	1000.0	ml

PROCEDURE

Use standard procedures to obtain isolated colonies from specimens. 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. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

EXPECTED RESULTS

Typical colonial morphology on EMB Agar is as follows:

Escherichia 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

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 ± 2°C for 24 hours	Growth
Proteus vulgaris ATCC 9484	35 ± 2°C for 24 hours	Growth
Shigella flexneri ATCC 12022	35 ± 2°C for 24 hours	Growth

BIBLIOGRAPHY

1. Endo. 1904. Zentralbl. Bakteriol., Abt. I Orig. 35:109.

2. Holt-Harris and Teague. 1916. J. Infect. Dis. 18:596.

3. Levine. 1918. J. Inf. Dis. 23:43.