TECHNICAL DATA SHEET

EMB AGAR (LEVINE)

CONFIRMATION OF *ESCHERICHIA COLI*

1 INTENDED USE

EMB Agar, originally recommended by Levine, is used to isolate and identify enterobacteria (notably *Escherichia coli* and *Enterobacter aerogenes*) in pharmaceutical, cosmetic and food products as well as water.
It is also used as a confirmation media for *Escherichia coli* in cosmetic products.

The typical composition reflects that defined in the standard NF EN ISO 21150.

2 HISTORY

In 1916, Holt-Harris and Teague used the combination of eosin and Methylene blue to differentiate microorganisms as a function of whether or not they could ferment lactose. Levine subsequently modified the formula by removing sucrose and increasing the lactose concentration, which led to the easy differentiation between *Escherichia coli* and *Enterobacter aerogenes*.

3 PRINCIPLES

Eosin Y and Methylene blue have low selective capacities, since they only partially inhibit the development of Gram-positive bacteria such as enterococci.

The dyes allow to the differentiation between lactose-positive and lactose-negative bacteria. Coliform strains form violet to brown colonies, while salmonellae are colorless, transparent or amber.
Escherichia coli present a metallic sheen under oblique lighting.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- 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
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25 °C : 7,1 ± 0,2.

5 PREPARATION

- Dissolve 37,5 g of dehydrated media (BK056) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the medium at 44-47 °C.
- Mix well to oxidize the methylene blue and insure the homogeneous suspension of the precipitate.
- Pour into sterile Petri plates and let solidify on a cool, flat surface.
- Dry in an incubator with the covers partially removed.

✓ **Reconstitution : 37,5 g/L**

✓ **Sterilization : 15 min at 121 °C**

6 INSTRUCTIONS FOR USE

Confirmation of *Escherichia coli*, Cosmetics (NF EN ISO 21150)

- Inoculate a characteristic colony on MacConkey agar and isolate by streaking onto EMB agar prepared as above.
- Incubate at 30-35 °C for 24 to 48 hours.

✓ **Inoculation :**
Surface

✓ **Incubation :**
24 to 48 h at 30-35°C

NOTE
For other applications, incubate generally for 18 to 24 hours at 37 ± 1 °C.

7 RESULTS

Colonies have the following appearance :

Characteristics	Microorganisms
Dark violet colonies, convex, low confluence, 2-3 mm in diameter with a black center reaching more than 3/4 of the diameter and which exhibit a greenish metallic sheen in reflected light and a blue-black aspect under direct light	*Escherichia coli*
Bluish flattened colonies, relatively confluent, 4-6 mm in diameter with a dark brown center, occasionally with a metallic sheen	*Enterobacter aerogenes*
Violet colonies with slight metallic sheen	*Citrobacter*
Brownish mucous colonies	*Klebsiella*
Transparent amber colonies	*Salmonella* et *Shigella*

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : violet powder, free-flowing and homogeneous.
Prepared media : claret-colored agar, which may contain a slight precipitate after autoclaving.

Typical culture response after 24 hours of incubation at 30-35 °C (NF EN ISO 21150) :

Microorganisms		Growth	Characteristics
Escherichia coli	WDCM 00012	Good, score 2	violet colonies with greenish metallic sheen

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in vials (*) : 180 days at 2-8 °C.
Prepared media in plates (*) : 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK056HA

11 BIBLIOGRAPHY

Holt-Harris, J.E., and Teague, O. 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis., 18: 596.

Levine, M. 1918. Differentiation of *Escherichia coli* and *Aerobacter aerogenes* on a simplified eosin-methylene blue agar. Jour. Inf. Dis., 23: 43-47.

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Weld, J.T. 1952. *Candida albicans*. Rapide identification in pure cultures with carbon dioxyde on modified eosin methylene blue medium. Arch. Dermat. Syph., 66: 691-694.

Vogel, R.A., and Moses, M.R. 1957. Welds method for the rapid identification of *Candida albicans* in clinical materials. Am. J. Clin. Pathol., 28 (1): 103.

NF EN ISO 21150. Septembre 2009. Cosmétiques. Microbiologie. Détection d'*Escherichia coli*.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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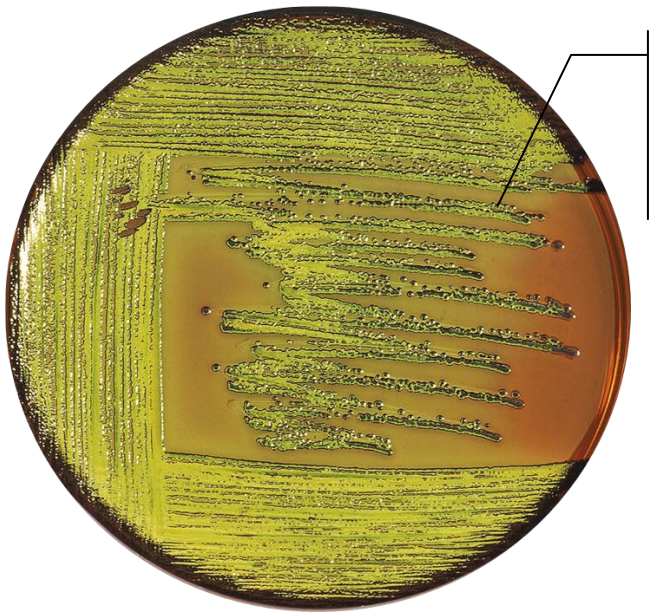
ANNEXE 1 : SUPPORT PHOTO

EMB Agar (Levine)

Detection of *E. coli* and *Enterobacter*

Results :

Growth obtained after 24 hours of incubation at 37°C.



Escherichia coli

Characteristic colonies :
Dark violet color, concave, presenting a green metallic sheen under reflected light.