LaMotte BioPaddles™ TECH DOCUMENT
Call: 800-344-3100
Email: tech@lamotte.com

MAC/EMB

Code 5544

MacConkey Agar (**MAC**)
Eosin Methylene Blue Agar (**EMB**)

USE:

Isolation and differentiation of Gram (-) enteric bacilli (**MAC**) / Coliform Testing / Recovery of Stressed Coliforms (**EMB**)

Side 1: MacConkey Agar (**MAC**) (pink)
(Side 1 is marked with an indented laser line)

Side 2: Eosion Methylene Blue Agar (**EMB**) (dark red)

APPLICATION

Enumeration of Gram (-) rods, in particular, coliforms, and specifically, *E. coli*.

PADDLE AGARS

MacConkey Agar (MAC) – Both selective and differential; used to differentiate between Gram negative bacteria while inhibiting the growth of most Gram positive bacteria. The medium also differentiates between lactose-fermenting coliforms Lac (+) and lactose non-fermenters Lac (-), which include potential pathogens. Addition to the nutrient agar base of bile salts and crystal violet will inhibit the growth of most Gram positive bacteria, making MacConkey agar selective. Lactose, a fermentable carbohydrate, and neutral red, a pH indicator, are added to differentiate the lactose positive coliforms from the potentially pathogenic lactose non-fermenters. When lactose is fermented, acid products lower the pH below 6.8, with the resulting colonial growth turning pinkish-red. If an organism is unable to ferment lactose, the colonies will be colorless. Bile salts mixture and crystal violet are the selective agents, inhibiting Gram positive cocci and allowing Gram negative organisms to grow. Sodium Chloride maintains the osmotic environment. Agar and a proprietary polymer are the solidifying agents.

Eosin Methylene Blue Agar-Levine (EMB) - Differential and slightly selective medium for the isolation, cultivation and differentiation of Gram negative enteric microorganisms (bacilli) isolated from both clinical and nonclinical specimens[1]. Gram (+) bacteria are inhibited. It is widely used for the examination of materials for the presence of coliforms. Eosin Methylene Blue Agar, abbreviated EMB, was developed by Holt-Harris and Teague.[2] This formula contains lactose and sucrose with two indicator dyes, Eosin Y and Methylene Blue. Levine modified the formula by removing sucrose and doubling the concentration of lactose.[3] [4]

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[1] Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

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

[3] Levin, M. 1918. Differentiation of E. coli and A. aerogenes on a simplified eosin-methylene blue agar. J. Infect. Dis. 23:43-47.

[4] Levin, M. 1921. Bacteria fermenting lactose, the significance in water analysis. Bull. 62. Iowa State College Eng. Exp. Sta., Ames, Iowa.

NOTE: EMB Agar is moderately inhibitory. Some staphylococci, streptococci and yeast may grow. They will appear as small, pinpoint colonies. Not all strains of *E. coli* produce a green metallic sheen. The presence of the green metallic sheen is not diagnostic for *E. coli*.[5]

CULTURE CONTROLS

10-300 inoculum (CFU)

	MAC Agar	**EMB Agar**
Enterococcus faecalis	INHIBITED	PARTIAL INHIBITION (colorless colonies)
Escherichia coli	GROWTH	GROWTH (blue-black w/ green metallic sheen)
Proteus mirabilis	GROWTH (partial inhibition of swarming)	GROWTH
Salmonella typhimurium	GROWTH	GROWTH

STORAGE / EXPIRATION

Store tightly sealed BioPaddles™ in a cool, dry location (less than 68°F/20°C). Avoid temperature changes. BioPaddles™ may be refrigerated, but it is not necessary. Do not freeze. If freezing occurs, thaw (3-6 hours) under refrigeration temperatures (40°F; 4.4°C). Freezing can promote excess water loss and variation in media surface due to crystal formation. The average shelf-life is one year. Refer to expiration date (See: BBE stamped on vial). Discard if paddle agar appears oxidized (darkens from expected color). The expiration date applies to the medium in an intact container when stored as directed.

SAMPLING

Liquids: Twist to remove paddle from vial. Fill vial to 40 mL fill line with the liquid to be sampled. The 40 mL volume can be used to calculate Total Viable Count (TVC) and/or Total Colony Count (TCC). Replace paddle. Allow a contact time of 15 seconds. Remove the paddle. Empty the vial. Replace the paddle in the vial.

Surfaces: Twist to remove paddle from vial. Allow the paddle surface (10 cm^2) to come into physical contact with the test surface. Recovery rate is about 50%. To insure an accurate recovery, gently sweep (or touch) the paddle to cover a 20 cm^2 area. Replace paddle in vial

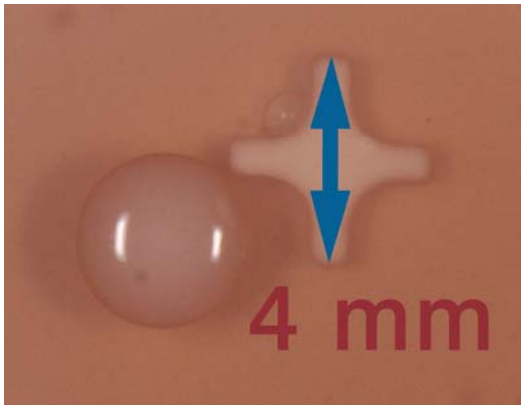
INCUBATION

Incubate at 35°C ±2°C for 18-24 hours.

[5] MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

COLONY MEASURING

Each BioPaddles™ paddle has molded media attachment points that are 4mm in length (point-to-point). This feature provides a useful guidepost to estimating nearby colony size.



IDENTIFICATION

ORGANISM		MAC			EMB		
ORGANISM	**PHYSIOLOGY ◆Precision Test Strip Available**	**GROWTH**	**COLONY**	**IMAGE**	**GROWTH**	**COLONY**	**IMAGE**
Bacillus spp.	• Lactose (-) • Indole (-) • Oxidase (-) • Catalase (+) • Gram (+) Rod	INHIBITED	---	---	INHIBITED	---	---
Candida albicans	• Catalase (+) • Actinomycetes	+++	• Cream • CVEG • 1-2mm		INHIBITED	---	---
E. coli	• Lactose (+) • Indole (+) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod)	+++	• Pink / Red • CVEG • 2-4mm		+++	• Blue-black bulls eye w/ green metallic sheen • CVEG • 2-4mm	

ORGANISM		MAC			EMB		
ORGANISM	**PHYSIOLOGY ◆Precision Test Strip Available**	**GROWTH**	**COLONY**	**IMAGE**	**GROWTH**	**COLONY**	**IMAGE**
Enterobacter aerogenes	• Lactose (+) • Indole (+) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Colorless • Thick, round, raised to low-convex; Spreading • 2-3mm		+++	• Pink to purple colonies often with a central dark purple dot (bulls eye); mucoid • CVEG • 2-4mm	**IMAGE PENDING**
Enterococcus spp.	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (-) ◆ • Urease (-) ◆ • Gram (+) Sphere	**PARTIAL - COMPLETE INHIBITION**	---	---	**PARTIAL - COMPLETE INHIBITION**	---	---
Klebsiella spp.	• Lactose (+) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (+) ◆ • Gram (-) Rod	+++	• Colorless / Light Pink • Spreading • 4-5mm		+++	• Pink to purple colonies often with a central dark brown dot (bulls eye); mucoid • CVEG • 4-5mm	**IMAGE PENDING**
Proteus spp.	• Lactose (-) • SEE: INDOLE ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (+) ◆ • Gram (-) Rod	+++	• Colorless; Spreading / swarming • 2-3mm		+++	• Colorless • CVEG • 0.5-1mm	
Pseudomomas aeruginosa	• Lactose (-) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod • Fluoresces blue under long-wave UV light (400-nm)	+++	• Cream / off-white • Irregular / spreading • Diffusible green-blue pigment • 2-4mm	**IMAGE PENDING**	+++	• Colorless • Irregular / spreading / glossy • 2-4mm	**IMAGE PENDING**

LaMotte_BioPaddles_MAC_EMB

ORGANISM		MAC			EMB		
ORGANISM	**PHYSIOLOGY ◆Precision Test Strip Available**	**GROWTH**	**COLONY**	**IMAGE**	**GROWTH**	**COLONY**	**IMAGE**
Pseudomomas fluorescens	• Lactose (-) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod • Fluoresces blue-green under long-wave UV light (400-nm)	+++	• Colorless to pink • Center target • Glossy • Umbonate • 2-4mm		++	• Colorless to pink • Center target • Glossy • Umbonate • 2-4mm	
Salmonella typhimurium	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Clear, transparent or amber • Circular, Smooth, Flat • 2-3mm	IMAGE PENDING	++	• Colorless to gray, circular • FED • 2-4mm	IMAGE PENDING
Salmonella epidermidis	• Lactose (-) ◆ • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	PARTIAL - COMPLETE INHIBITION	---	---	++	• Colorless to gray, • Circular • Umbonate • 2-4mm	
Serratia spp.	• Lactose (-) ◆ • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (+) ◆ • Gram (-) Rod	++	• Pink / Red • 2-4mm	IMAGE PENDING	++	• Colorless • 1-3mm	MAGE PENDING
Shigella spp.	• Lactose (-) • Indole - mixed ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Colorless or transparent / faintly pink • Raised • 1-3mm	IMAGE PENDING	++	• Colorless to pink; circular • FED • 2-4mm	IMAGE PENDING

ORGANISM		MAC			EMB		
ORGANISM	**PHYSIOLOGY ◆Precision Test Strip Available**	**GROWTH**	**COLONY**	**IMAGE**	**GROWTH**	**COLONY**	**IMAGE**
Staphylococcus aureus	• Lactose (-) ◆ • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Gram (+) Sphere	PARTIAL - COMPLETE INHIBITION	---	---	PARTIAL - COMPLETE INHIBITION	---	---
Streptococcus spp.	• Lactose (+) • Indole (+) • Oxidase (-) • Catalase (-) • Gram (+) Sphere	PARTIAL - COMPLETE INHIBITION	---	---	PARTIAL - COMPLETE INHIBITION	---	---
Gram (+) Bacteria		PARTIAL - COMPLETE INHIBITION	---	---	PARTIAL - COMPLETE INHIBITION	---	---

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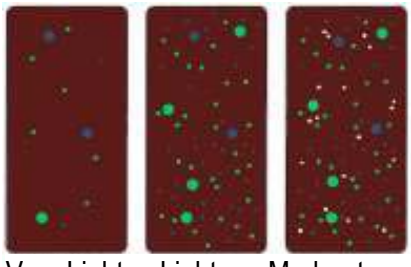
[6] MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

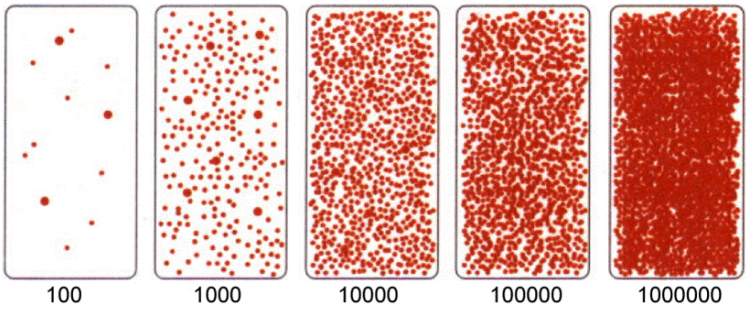
ENUMERATION

MAC

EMB



TVC / TCC



Approximate Colony Count per 100 mL

TVC/TCC
(Total Viable Count/ Total Colony Counts)

Colony Counts <1000
Count colonies
TVC/TCC Count = Count x 2.5

Colony Counts >1000
Use chart
TVC/TCC Count = Count x 2.5

(Based on a 40 mL sample)

Example:
Inoculated NUT/TTC paddle showing approximately 1000 CFU/100 mL.

DISPOSAL

Twist to remove paddle from vial. Fill vial to 40 mL fill line with 1:9 dilution of household bleach (5.25% sodium hypochlorite). Replace paddle in vial. Allow 15 minute contact time. Remove paddle. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen cap and microwave for 30 seconds, autoclave, or incinerate.

.

GLOSSARY:

CVEG	Convex, Entire, Glossy
FED	Full, Entire, Dull
Catalase	Cat (+) contains catalase enzymes that degrade cellular H_2O_2.
Lactose	Lac (+) bacteria can ferment available lactose in the medium producing an acid which lowers the pH. Lac (-) are non-fermenting.
Indole	Biochemical test to determine the ability of an organism to split indole from the amino acid tryptophan. P. vulgaris is indole (+) while P. mirabilis is indole.
Oxidase	OX (+) contains cytochrome c oxidase. In contact with an indicator turns dark blue if OX (+); colorless if OX(-).
Urease	UR (+) presence of enzyme urease which hydrolyzes urea into CO_2 & NH_4.
β-D-Glucoronidase	Glu (+) Found in 97% of E. coli strains. The presence of E. coli is determined when both β-D-Glucoronidase and Indole are (+), and the organism is Gram (-).
Gram	Gram reaction