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MAC/EMB MICROSLIDE®

TECHNICAL DOCUMENT

Index

MAC/EMB

CODE: M-MAC/EMB

USE

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

APPLICATION

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

PADDLE AGARS

Side 1: MacConkey Agar (MAC) – (Color: Watermelon) 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.

Side 2: Eosin-Methylene Blue Agar (EMB) – (Color: Maroon) Differential (and slightly selective) medium for the isolation, cultivation and differentiate of Gram-negative enteric microorganisms (bacilli). Gram-positive bacteria are inhibited.

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.

***Note:** Side 1 of each paddle is marked with an indented laser line.

STORAGE / EXPIRATION

Microslides® should be stored tightly sealed (unopened) in a cool, dry location at room temperature (18 - 25°C; 65 - 77°F). Temperature fluctuations may result in condensation settling at the bottom of the vial, although this does not affect culture properties, it could reduce the shelf-life or cause the agar to separate from the plastic paddle support. Refer to 'Best Before End date' (SEE: BBE stamped on vial).

Avoid sudden temperature changes. Shield from direct sunlight. Do not allow paddles to freeze. Do not store in a refrigerator (~44°F / 10°C) or at temperatures exceeding 80°F; 27°C. Refrigeration may result in water condensation. Discard if paddle agar appears oxidized (darkened from expected color) or if contaminants appear. Expiry applies to medium in its intact container when stored as directed.

AGAR VERIFICATION

These agars have been verified by EMSL Analytical, Inc. using *E. coli* and *E. faecalis* cultures.
Documentation available upon request.

SAMPLING

SURFACE Sampling Protocol

1. Remove the paddle from the vial. Do not touch the agar surfaces.
2. To assure an accurate area recovery, contact the paddle to 20^2cm of the surface by contacting the surface twice in separate 10^2cm areas.
3. Replace paddle in vial.
4. Incubate.

LIQUID Sampling Protocol

DIRECT IMMERSION PROTOCOL – low viscous liquids

1. Mix liquid test sample.
2. Remove the paddle from the vial. Do not touch the agar surfaces.
3. When taking the sample:
    a. Pour 40mL of the sample into the vial (to the printed horizontal fill line; see right). Dip the paddle into the 40mL volume liquid in the vial. Maintain a contact time of at least 15 seconds (30 seconds optimal). Both agar surfaces must be completely contacted.

    b. Or dip the paddle into the sample directly. Maintain a contact time of at least 15 seconds (30 seconds optimal). Both agar surfaces must be completely contacted.
4. Allow excess fluid to drain off both paddle agar surfaces.
5. Replace paddle in vial.
6. Incubate.

SPREAD Protocol – high viscous liquids

1. Mix liquid test sample.
2. Remove paddle from vial. Do not touch the agar surfaces.
3. Holding the contact agar surface on a horizontal plane, deposit volume as a single drop approximately 1cm from the handle boundary (Figure 1).
4. Position a sterile glass rod on the "handle" side of the drop and bring it into contact with the drop creating a meniscus. Drag the glass tube over the paddle agar surface.
5. Replace paddle in vial.
6. Incubate.

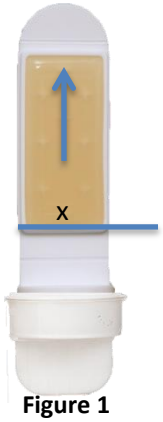


Figure 1

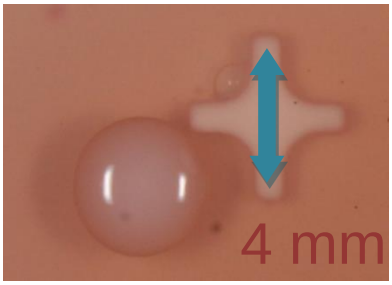
INCUBATION

Incubation of Paddle Growth	Incubation Temperature	Examine at:
Yeast / Mold	25 to 30°C	48 hours up to 120 hours (5 days)
Yeast / Mold	Room Temperature	Up to 7 days
Total Coliform / Bacteria	35 ± 2°C	24 to 48 hours
Total Coliform / Bacteria	Room Temperature	Up to 5 days

Note: Incubation of bacteria after 48 hours may produce confluent growth making enumeration more difficult.

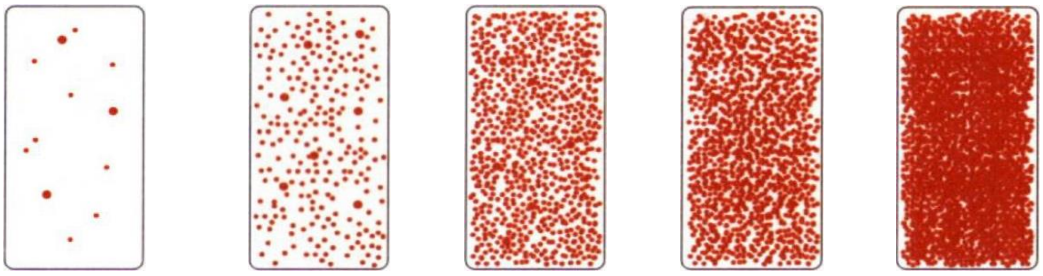
COLONY MEASURING

Each Microslide® 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.



ENUMERATION

Bacteria CFU/mL



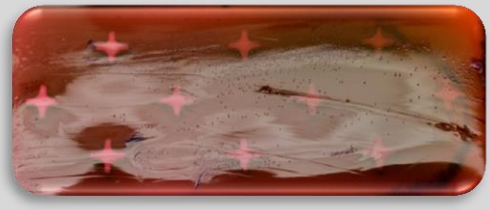
10^3 cfu/mL	**10^4 cfu/mL**	**10^5 cfu/mL**	**10^6 cfu/mL**	**10^7 cfu/mL**
(1,000)	(10,000)	(100,000)	(1,000,000)	(10,000,000)
(Light)		**(Moderate)**		**(Heavy)**

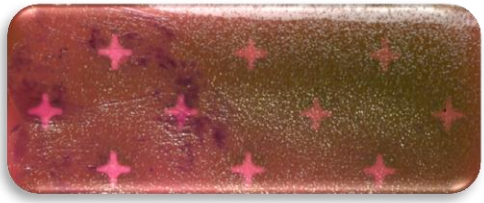
Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold growth and surface testing.

DISPOSAL

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

IDENTIFICATION

Organism	MacConkey (MAC)	Eosin-Methylene Blue (EMB)
Bacillus spp.	INHIBITED	INHIBITED
Candida albicans	INHIBITED	INHIBITED
E. coli	 Growth: +++ Colony: Pink/Red, CVEG, 0.2-0.5mm	 Growth: +++ Colony: Blue-black bulls-eye w/ green-metallic sheen, CVEG, 2-4mm
Enterobacter aerogenes	 Growth: +++ Colony: Colorless, thick, round, raised to low-convex, spreading, 0.1-0.5mm	 Growth: +++ Colony: Pink/purple, dark purple bulls-eye, mucoid, CVEG, 0.1-0.5mm
Enterococcus spp.	PARTIAL TO COMPLETE INHIBITION	PARTIAL INHIBITION
Klebsiella spp.	 Growth: +++ Colony: Colorless/light pink, spreading, 0.5-1.0mm	 Growth: +++ Colony: Pink/purple, dark purple bulls-eye, mucoid, CVEG, 0.5-1.0mm
Proteus spp.	 Growth: + Colony: Colorless to yellow, pink/red, circular, wrinkled (flower-like), umbonate, erose, 2-4mm	 Growth: +++ Colony: Maroon/red, CVEG, 0.1-0.2mm (punctiform)

Pseudomonas aeruginosa	 Growth: +++ Colony: Transparent, CVEG, 0.1-0.2mm	 Growth: +++ Colony: Transparent with green metallic sheen on agar surface, CVEG, 0.01-0.1mm (punctiform)
Pseudomonas fluorescens	 Growth: +++ Colony: Colorless/Pink, center target, glossy, umbonate, 2-4mm	 Growth: ++ Colony: Colorless/Pink with darker center, umbonate, glossy, undulate, 2-4mm+
Salmonella typhimurium	 Growth: ++ Colony: Gray to white (pearl), circular, umbonate, 1-2mm	 Growth: ++ Colony: Maroon (red), punctiform; slight green metallic sheen, 0.1-0.5mm
Salmonella enteriditis	 Growth: +++ Colony: Gray to white (pearl), circular, umbonate, entire, 1-2mm	 Growth: +++ Colony: Maroon (red), slight green metallic sheen, FED, 0.01-0.1mm (punctiform)
Serratia spp.	 Growth: + Colony: Pink, convex, dull, entire, 0.1-	 Growth: +++ Colony: Transparent with a metallic green

	0.5mm (punctiform)	sheen, circular to irregular, raised to convex, glossy, entire, 0.01-0.5mm (punctiform)
Shigella spp.	 Growth: +++ Colony: Transparent to gray (pearl), circular, raised, dull, entire, 1-2mm	 Growth: ++ Colony: Transparent to milky white center with transparent boarder, irregular, mucoidal, raised, glossy, undulate, 1-2mm
Staphylococcus aureus	PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION
Streptococcus spp.	 Growth: + Colony: Transparent, circular, umbonate, glistening, entire, 1-2mm	PARTIAL TO COMPLETE INHIBITION
Gram (+) bacteria	PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION

GLOSSARY

CVEG ..... Convex, Entire, Glossy
FED ..... Full, Entire, Dull
Gram ..... Gram reaction