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NUT/MAC MICROSLIDE® TECHNICAL DOCUMENT

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Index

NUT/MAC

CODE: M-NUT/MAC

USE

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

APPLICATION

In total coliform testing (TCC), the coliform organisms tested for include: total coliform, fecal coliform, and E. coli (*Escherichia coli*). Detection of fecal coliforms (a subset of total coliforms) or *Escherichia coli* (a subset of fecal coliforms) can indicate the potential presence of waterborne pathogens associated with fecal contamination[1].

PADDLE AGARS

Side 1: Nutrient-TTC Agar (NUT) – (Color: Yellow) General purpose (relatively non-selective) medium, which will support the growth of a wide variety of organisms. Suitable for cultivation of both aerobes and anaerobes. Aerobic coliform bacteria can be detected by their ability to reduce the TTC dye to a red-colored formozan dye. Bacterial colonies appear as red dots on an otherwise yellow medium.

Note: Paddle color is normally LIGHT YELLOW when the NUT agar is cast (about pH 6.0). Some microorganism growth (even before colonies are OBSERVABLE) will shift the pH from an acidic to a more alkaline level (pH 7.0 or higher) – turning the agar a light green.

Side 2: 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.

***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.

[1] United States Pharmacopeial Convention. 2007. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.

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.

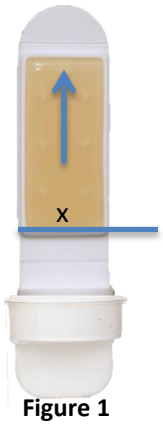


Figure 1

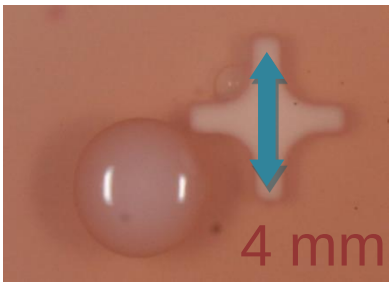
INCUBATION

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.

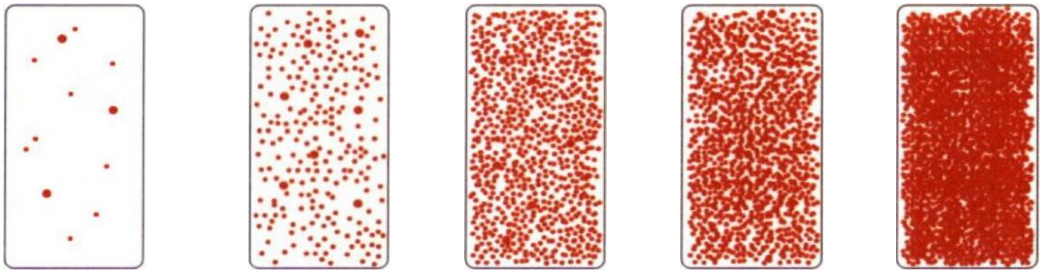
COLONY MEASURING

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)**

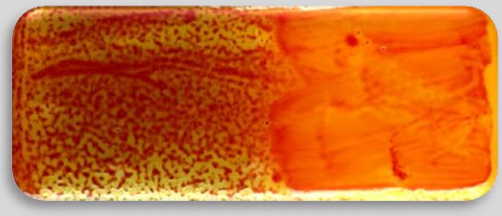
Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold growth and surface testing.

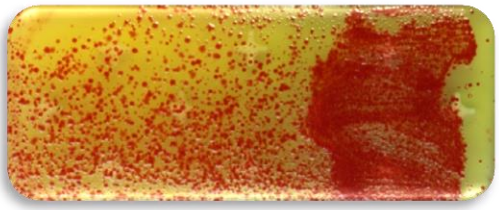
DISPOSAL

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	Nutrient-TTC (NUT)	MacConkey (MAC)
Aspergillus niger	 Growth: +++ Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++cm	INHIBITED
Bacillus spp.	 Growth: +++ Colony: Opaque with dark center (bullseye), irregular, raised, lobate (wrinkled), 2-4mm+	INHIBITED
Candida albicans	 Growth: +++ Colony: Cream, CVEG, 1-2mm	INHIBITED
E. coli	 Growth: +++	 Growth: +++

	Colony: Yellow/Orange/Red, CVEG, 2-4mm	Colony: Pink/Red, CVEG, 0.2-0.5mm
Enterobacter aerogenes		
	Growth: +++ Colony: Maroon/red with transparent margin, CVEG, 0.1-0.5mm	Growth: +++ Colony: Pink, thick, round, raised to low-convex, spreading, 0.1-0.5mm
Enterococcus spp. *Klebsiella spp.*	INHIBITED	PARTIAL TO COMPLETE INHIBITION
		
	Growth: +++ Colony: Amber/Red, spreading, 0.5-1.0mm	Growth: +++ Colony: Colorless/light pink, spreading, 0.5-1.0mm
Proteus spp.		
	Growth: +++ Colony: Maroon/red with dark red center and transparent margin, irregular, glistening (swarming-transparent field), raised, undulate, 1-4mm	Growth: + Colony: Colorless to yellow, pink/red, circular, wrinkled (flower-like), umbonate, erose, 2-4mm
Pseudomonas aeruginosa		
	Growth: +++ Colony: Maroon/red with transparent margin, circular to irregular, raised, entire, 1-2mm	Growth: +++ Colony: Transparent, CVEG, 0.1-0.2mm (punctiform)

Pseudomonas fluorescens	 Growth: +++ Colony: Clear/colorless with grey/dark center, translucent edges, irregular/spreading to confluent, 2-4mm	 Growth: +++ Colony: Clear/pink with dark pink center, transluecent edges, irregular edges, 2-4mm
Salmonella enteriditis	 Growth: +++ Colony: Red, FED, 0.5-1.0mm	 Growth: +++ Colony: Gray to white (pearl), circular, umbonate, entire, 1-2mm
Serratia spp.	 Growth: ++ Colony: Red, FED, 0.5-1.0mm	 Growth: + Colony: Pink, convex, dull, entire, 0.1-0.5mm (punctiform)
Shigella spp.	 Growth: + Colony: Maroon/red, CVEG, 0.5-1.0mm	 Growth: +++ Colony: Transparent to gray (pearl), circular, raised, dull, entire, 1-2mm
Staphylococcus aureus	 Growth: +	PARTIAL TO COMPLETE INHIBITION

Streptococcus spp.	Colony: Red, FED, 0.5-1.0mm	
		
	Growth: ++ Colony: Maroon/red, CVEG, 0.1-0.5mm	Growth: + Colony: Transparent, circular, umbonate, glistening, entire, 1-2mm
Streptomyces griseus		PARTIAL TO COMPLETE INHIBITION
	Growth: + Colony: Yellow, FED, 0.5-1.0mm	
Gram (+) Bacteria	PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION

GLOSSARY

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