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# NUT/MAC

## MICROSLIDE® TECHNICAL

## DOCUMENT



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# 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<sup>1</sup>.

## 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:** The Nutrient-TTC agar color is normally light yellow when the agar is cast. After testing, during the incubation phase, the agar may change to a light green color. This color change is a result of either a microbial-induced or chemically-induced pH change in the media. This color change alone does not indicate the presence of microorganisms. Development of red spots or other growth on the agar are an indication of microorganisms.

**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

<sup>1</sup> United States Pharmacopeial Convention. 2007. The United States pharmacopeia, 31<sup>st</sup> ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.

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<sup>2</sup>cm of the surface by contacting the surface twice in separate 10<sup>2</sup>cm 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.

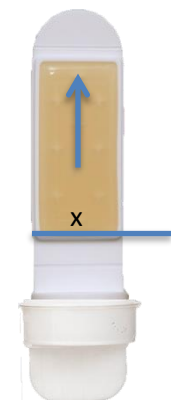


Figure 1

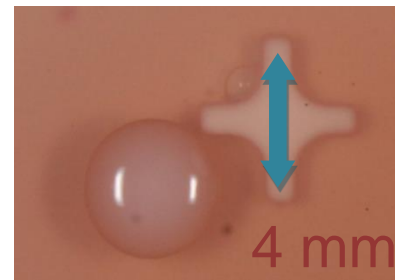
## INCUBATION

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.

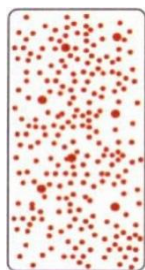


## ENUMERATION

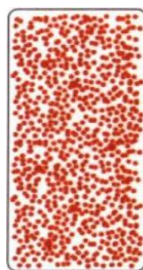
### Bacteria CFU/mL



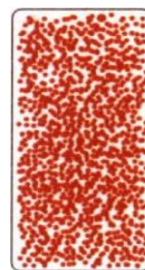
**10<sup>3</sup> cfu/mL**  
(1,000)  
**(Light)**



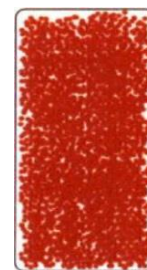
**10<sup>4</sup> cfu/mL**  
(10,000)



**10<sup>5</sup> cfu/mL**  
(100,000)  
**(Moderate)**



**10<sup>6</sup> cfu/mL**  
(1,000,000)




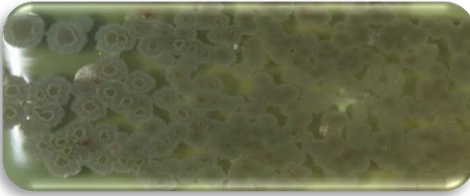
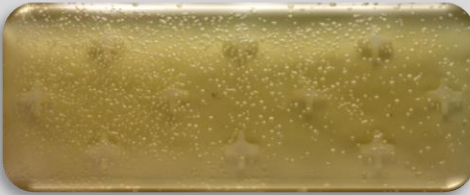
**10<sup>7</sup> cfu/mL**  
(10,000,000)  
**(Heavy)**


**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)
<i>Aspergillus niger</i>	 <p>Growth: +++ Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++cm</p>	INHIBITED
<i>Bacillus spp.</i>	 <p>Growth: +++ Colony: Opaque with dark center (bullseye), irregular, raised, lobate (wrinkled), 2-4mm+</p>	INHIBITED
<i>Candida albicans</i>	 <p>Growth: +++ Colony: Cream, CVEG, 1-2mm</p>	INHIBITED

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



*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, translucent edges, irregular edges, 2-4mm

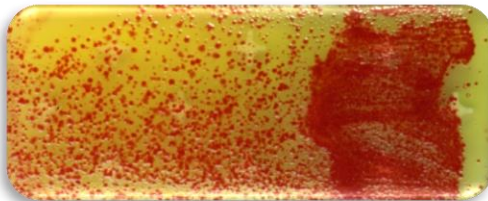
*Salmonella enteritidis*



Growth: +++  
Colony: Red, FED, 0.5-1.0mm

Growth: +++  
Colony: Gray to white (pearl), circular, umbonate, entire, 1-2mm

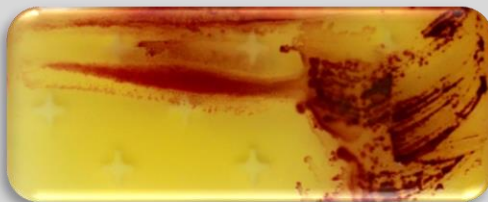
*Serratia spp.*






Growth: ++  
Colony: Red, FED, 0.5-1.0mm

Growth: +  
Colony: Pink, convex, dull, entire, 0.1-0.5mm (punctiform)

*Shigella spp.*





<i>Staphylococcus aureus</i>	Growth: + Colony: Maroon/red, CVEG, 0.5-1.0mm	Growth: +++ Colony: Transparent to gray (pearl), circular, raised, dull, entire, 1-2mm PARTIAL TO COMPLETE INHIBITION
		
<i>Streptococcus spp.</i>	Growth: + 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 PARTIAL TO COMPLETE INHIBITION
<i>Streptomyces griseus</i>		
<i>Gram (+) Bacteria</i>	Growth: + Colony: Yellow, FED, 0.5-1.0mm PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION

## GLOSSARY

<b>CVEG</b> .....	Convex, Entire, Glossy
<b>FED</b> .....	Full, Entire, Dull
<b>Gram</b> .....	Gram reaction