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NUT/MALT MICROSLIDE® TECHNICAL DOCUMENT



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NUT/MALT

CODE: M-NUT/MALT

USE

Isolation and differentiation of Gram (-) enteric bacilli. Coliform Testing / Recovering of Stressed Coliforms (**NUT**). Optimal growth of molds and yeasts while restricting bacterial growth. (**MALT**).

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 contamination¹. Malt Agar is used for the examination of yeasts and molds while restricting bacterial growth.

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: Malt Extract Agar (MALT) – (Color: Cream) The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

***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

¹ United States Pharmacopeial Convention. 2007. The United States pharmacopeia, 31st 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* (NUT) and *P. commune* and *C. albicans* (MALT) 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 20²cm of the surface by contacting the surface twice in separate 10²cm 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.

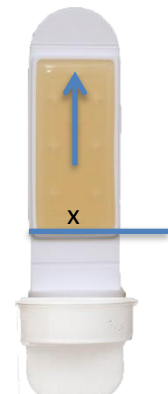


Figure 1

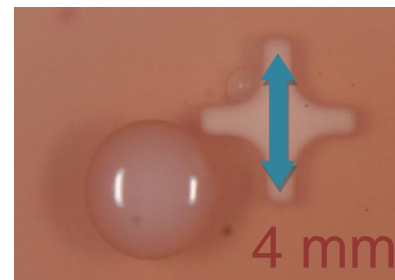
INCUBATION

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.

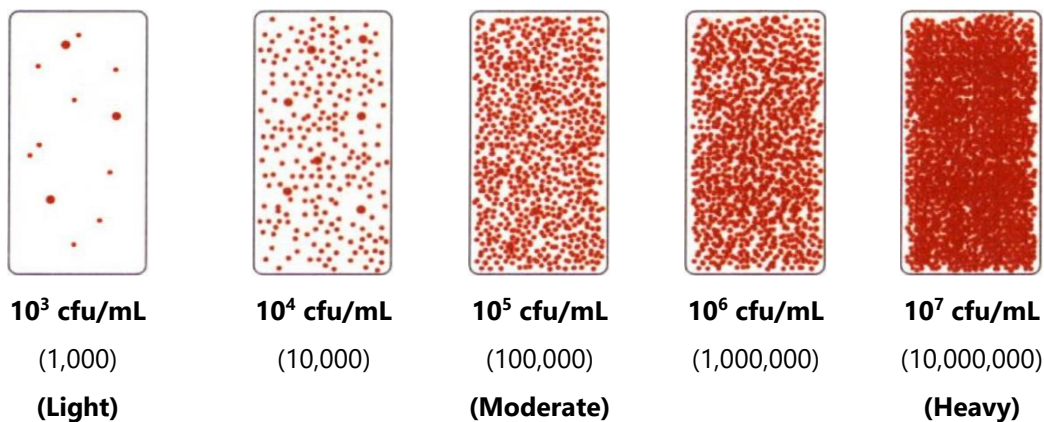
COLONY MEASURING

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





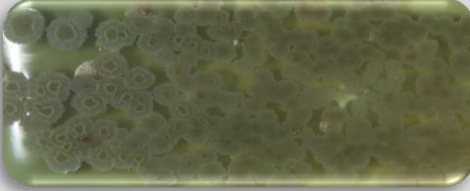

Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold growth and surface testing.

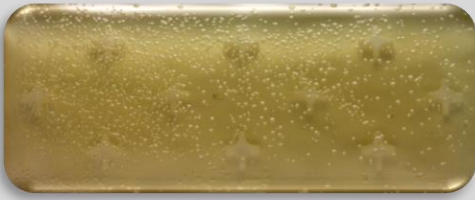





DISPOSAL

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)	Malt Extract (MALT)
<i>Actinomyces bovis</i>	Growth: + Colony: Opaque/tan-grey, CVEG, 1-3mm	Growth: ++ Colony: Opaque/tan-grey, CVEG, 1-3mm
<i>Alternaria spp.</i>	Growth: + Colony: Downy to wooly; flat, grayish, short, aerial hyphae, later becomes greenish black or olive-brown with a light border, 3-9cm	Growth: ++ Colony: Downy to wooly; flat, grayish, short, aerial hyphae, later becomes greenish black or olive-brown with a light border, 3-9cm
<i>Aspergillus niger</i>		
	Growth: +++ Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++cm	Growth: +++ Colony: Granular, white with jet black fruiting bodies, yellow/grey hyphae
<i>Aspergillus flavus</i>	Growth: + Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm	Growth: +++ Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm
<i>Aspergillus fumigatus</i>	Growth: + Colony: Granular to cottony, blue-green, green-grey, or green-brown, 3-9cm	Growth: +++ Colony: Granular to cottony, blue-green, green-grey, or green-brown, 3-9cm
<i>Aspergillus terreus</i>	Growth: + Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm	Growth: +++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm
<i>Bacillus spp.</i>		
	Growth: +++ Colony: Opaque with dark center (bullseye), irregular, raised, lobate (wrinkled), 2-4mm+	Growth: Translucent to dull, off-white, smooth to rough, irregular, dendroid margins to spreading, 1-2mm
<i>Botrytis spp.</i>	Growth: + Colony: Wooly, white/grey/brown pigment, 3-9cm	Growth: +++ Colony: Wooly, white/grey/brown pigment, 3-5++cm

<i>Candida albicans</i>	 <p>Growth: +++ Colony: Cream, CVEG, 1-2mm</p>	 <p>Growth: +++ Colony: White/Cream, smooth, spreading, 6mm</p>
<i>Chaetomium spp.</i> <i>Cladosporium spp.</i>	<p>PARTIAL TO COMPLETE INHIBITION</p>  <p>Growth: + Colony: Granular to wooly (velvety), olive-brown to black/brown, sometimes grey on a dark base, 2-5++cm</p>	<p>Growth: +++ Colony: Wooly, white/grey/olive, 3-5cm</p>  <p>Growth: +++ Colony: Granular to wooly (velvety), olive-brown to black/brown, sometimes grey on a dark base, 2-5++cm</p>
<i>Epicoccum spp.</i>	<p>Growth: + Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm</p>	<p>Growth: +++ Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm</p>
<i>E. coli</i>	 <p>Growth: +++ Colony: Yellow/Orange/Red, CVEG, 2-4mm</p>	<p>PARTIAL TO COMPLETE INHIBITION</p>
<i>Enterobacter aerogenes</i>	 <p>Growth: +++ Colony: Maroon/red with transparent margin, CVEG, 0.1-0.5mm</p>	<p>PARTIAL TO COMPLETE INHIBITION</p>
<i>Enterococcus spp.</i>	<p>INHIBITED</p>	<p>PARTIAL TO COMPLETE INHIBITION</p>

Fusarium spp.

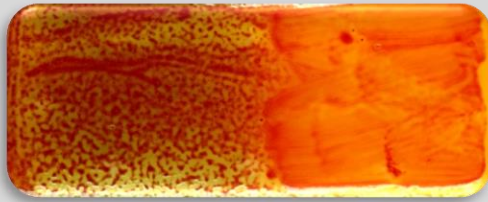


Growth: +
Colony: Woolly, flat, sometimes mucous-like



Growth: +++
Colony: Woolly, flat, sometimes mucous-like, white/yellow/pink, sometimes purple/brown pigment, 1-2cm
PARTIAL TO COMPLETE INHIBITION

Klebsiella spp.



Growth: +++
Colony: Amber/Red, spreading, 0.5-1.0mm

Microsporium spp.

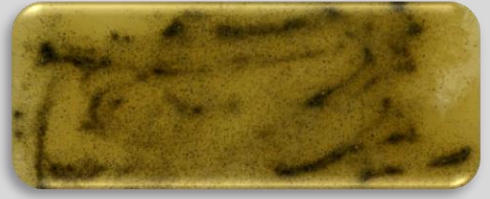
Growth: +
Colony: Glaborous (smooth), downy, wooly, powdery, white at first, later becoming grayish-yellow to blue-green with age, 1-9+cm

Growth: +
Colony: Glaborous (smooth), downy, wooly, powdery, white at first, later becoming grayish-yellow to blue-green with age, 1-9+cm

Mucor spp.



Growth: +
Colony: Woolly, fluffy (like cotton candy), white at first, later becoming gray/yellow to blue-green with age, 2-5++cm

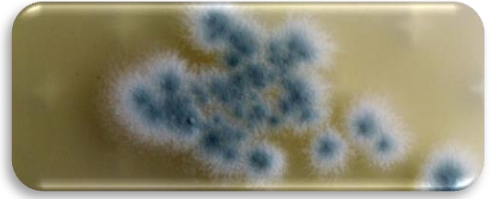


Growth: +
Colony: Woolly, fluffy (like cotton candy), white at first, later becoming gray/yellow to blue-green with age, 2-5++cm


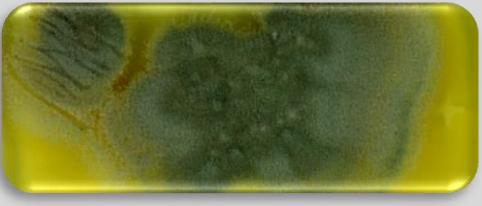



Penicillium chrysogenum (notatum)

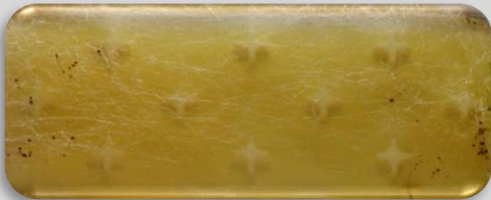




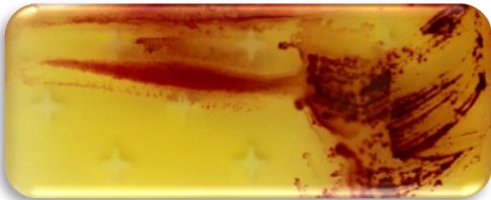







Growth: ++
Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 2-5++cm



Growth: ++
Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 2-5++cm

<i>Penicillium roqueforti</i>	 <p>Growth: + Colony: Granular, dull, green in colour, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm</p>	 <p>Growth: ++ Colony: Granular, dull, green in colour, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm</p>
<i>Penicillium digitum</i>	<p>Growth: + Colony: Woolly, fluffy (like cotton candy), white at first, later becoming green with age, 3-9cm</p>	<p>Growth: +++ Colony: Woolly, fluffy (like cotton candy), white at first, later becoming green with age, 3-9cm</p>
<i>Pithomyces spp.</i>	<p>Growth: + Colony: Powdery, pale/dark grey or brown pigment, 2-9++cm</p>	<p>Growth: +++ Colony: Powdery, pale/dark grey or brown pigment, 2-9++cm</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>PARTIAL TO COMPLETE INHIBITION</p>
<i>Pseudomonas aeruginosa</i>	 <p>Growth: +++ Colony: Maroon/red with transparent margin, circular to irregular, raised, entire, 1-2mm</p>	<p>PARTIAL TO COMPLETE INHIBITION</p>
<i>Pseudomonas fluorescens</i>	 <p>Growth: +++</p>	<p>PARTIAL TO COMPLETE INHIBITION</p>

<i>Rhizopus spp.</i>	Colony: Clear/colorless with grey/dark center, translucent edges, irregular/spreading to confluent, 2-4mm		
	Growth: +++ Colony: Cottony, white to black/grey (black fruiting bodies), 2-9++cm	Growth: +++ Colony: Cottony, white to black/grey (black fruiting bodies), 2-9++cm	
<i>Saccharomyces cerevisiae</i>			
	Growth: ++ Colony: Creamy white to tan, spreading, circular, entire, raised to convex, glistening surface, 5-8mm	Growth: +++ Colony: Creamy white to tan, spreading, circular, entire, raised to convex, glistening surface, 5-8mm	
<i>Salmonella typhimurium</i> <i>Salmonella enteritidis</i>	Growth: +++ Colony: Purple/pink, FED, 0.5-1.0mm	PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION
			
<i>Serratia spp.</i> <i>Shigella spp.</i>	Growth: +++ Colony: Red, FED, 0.5-1.0mm	PARTIAL TO COMPLETE INHIBITION	INHIBITED PARTIAL TO COMPLETE INHIBITION
			
	Growth: + Colony: Maroon/red, CVEG, 0.5-1.0mm		

<i>Staphylococcus aureus</i>		PARTIAL TO COMPLETE INHIBITION
	Growth: + Colony: Maroon/Red, FED, 0.5-1.0mm	
<i>Streptococcus spp.</i>		PARTIAL TO COMPLETE INHIBITION
	Growth: ++ Colony: Maroon/red, CVEG, 0.1-0.5mm	
<i>Streptomyces griseus</i>		PARTIAL TO COMPLETE INHIBITION
	Growth: + Colony: Yellow, FED, 0.5-1.0mm	
<i>Torula spp.</i>		
	Growth: + Colony: Arrowhead/circle or heart shape, red, 0.5-1.0mm	Growth: + Colony: Arrowhead/circle or heart shape, grey/white to brown with age, 3-9cm
<i>Trichoderma spp.</i>	Growth: ++ Colony: Cottony, white, later scattered green or yellow-green patches (rings), 2-9++cm	Growth: ++ Colony: Cottony, white, later scattered green or yellow-green patches (rings), 2-9++cm
	<i>Trichophyton spp.</i>	Growth: + Colony: Woolly with indented borders, white to brown/tan pigment, 2-9++cm
<i>Gram (+) Bacteria</i>	PARTIAL TO COMPLETE INHIBITION	Note: Low acidity inhibits the growth of most bacteria

GLOSSARY

CVEG.....Convex, Entire, Glossy

FED.....Full, Entire, Dull

Gram.....Gram reaction