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NUT/R2A

MICROSLIDE®

TECHNICAL DOCUMENT



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NUT/R2A

CODE: M-NUT/R2A

USE

Isolation and differentiation of Gram (-) enteric bacilli. Coliform Testing / Recovering of Stressed Coliforms (**NUT**). For bacterial plate counts of treated potable water as well as oligotrophic (thrive in low-nutrient conditions) heterotrophs. (**R2A**).

APPLICATION

(NUT) 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. R2A Agar, in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria.

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 formazan 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: R2A Agar (R2A) – (Color: Off-White) Used for bacterial plate counts of treated potable water, as well as oligotrophic (thrive in low-nutrient conditions) heterotrophs. R2A Agar, in combination with a lower incubation temperature and longer incubation time, stimulate the growth of stressed and chlorine-tolerant bacteria.

Note: Fast-growing bacteria may produce smaller size colonies on R2A Agar than on nutritionally rich media. R2A Agar is a low-nutrient medium intended for culturing compromised microorganisms.

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

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

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*, *E. faecalis* and *S. aureus* 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

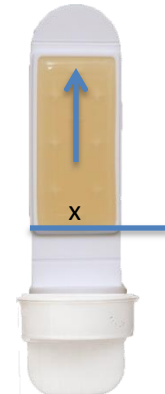
1. Mix liquid test sample.
2. Remove the paddle from the vial. Do not touch the agar surfaces.
3. When taking the sample:
 - a. Pour 40mL of the sample into the vial (to the printed horizontal fill line; see right). Dip the paddle into the 40mL volume liquid in the vial. Maintain a contact time of at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



SAMPLING (cont'd)

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.



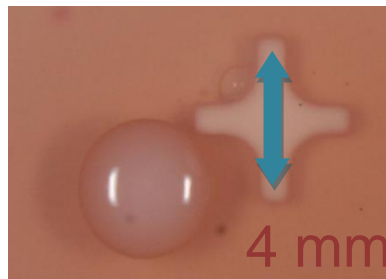
INCUBATION

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.

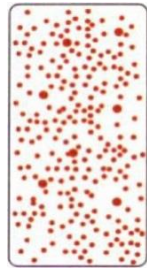


ENUMERATION

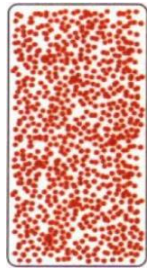
Bacteria CFU/mL



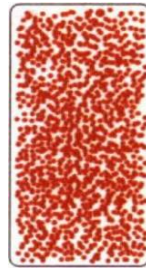
10³ cfu/mL
(1,000)
(Light)



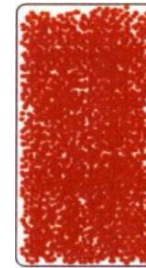
10⁴ cfu/mL
(10,000)



10⁵ cfu/mL
(100,000)
(Moderate)



10⁶ cfu/mL
(1,000,000)




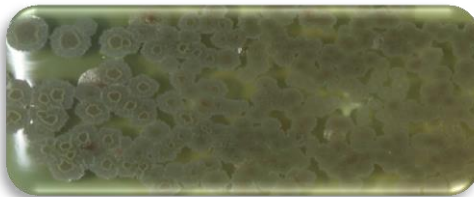

10⁷ cfu/mL
(10,000,000)
(Heavy)

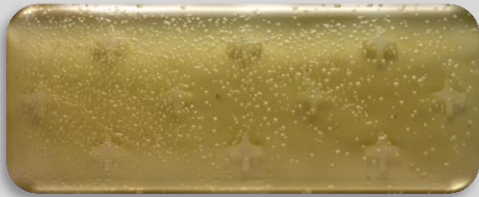

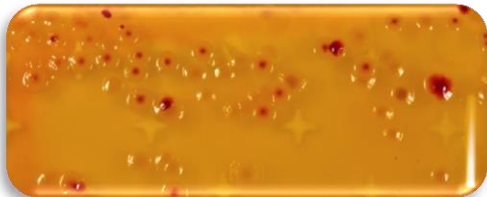




Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold growth and surface testing.

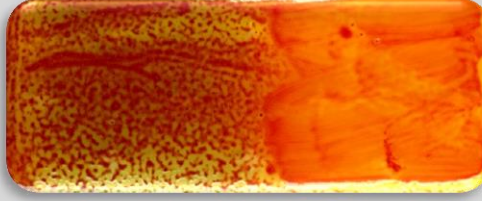




DISPOSAL


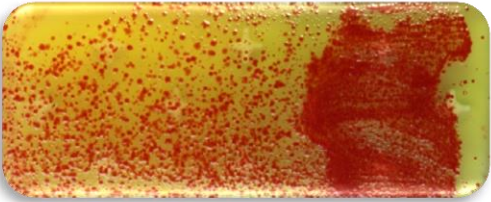

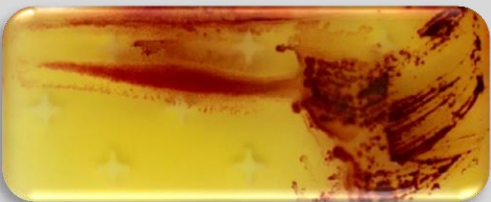

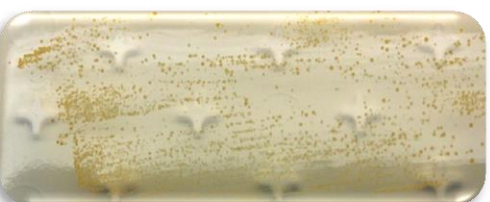


Make a 1:9 dilution of household bleach (5.25% sodium hypochlorite solution). Twist and remove Microslide[®] paddle from vial. Fill vial with 40mL diluted hypochlorite solution (to fill-line). Allow 15-minute contact time. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen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

IDENTIFICATION

Organism	Nutrient-TTC (NUT)	R2A
<i>Aspergillus niger</i>	 <p>Growth: +++ Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++cm</p>	PARTIAL TO COMPLETE INHIBITION
<i>Bacillus spp.</i>		

<i>Candida albicans</i>	Growth: +++ Colony: Opaque with dark center (bullseye), irregular, raised, lobate (wrinkled), 2-4mm+	Growth: +++ Colony: Translucent, dull, opaque, smooth to rough, irregular, dendroid margins to spreading, 2-4mm
		
<i>E. coli</i>	Growth: +++ Colony: Cream, CVEG, 1-2mm	Growth: +++ Colony: White, opaque, CVEG, spreading, 4-6mm
		
<i>Enterobacter aerogenes</i>	Growth: +++ Colony: Yellow/Orange/Red, CVEG, 2-4mm	Growth: ++ Colony: Translucent, CVEG, 0.5-1.0mm
		
<i>Enterococcus spp.</i>	INHIBITED	Growth: +++ Colony: Clear, CVEG, 0.05-0.2mm (punctiform)
		

<i>Klebsiella spp.</i>	 <p>Growth: +++ Colony: Amber/Red, spreading, 0.5-1.0mm</p>	 <p>Growth: +++ Colony: Transparent, circular to irregular, spreading, raised to slightly convex, entire to undulate, 0.5-1.0mm</p>
<i>Micrococci Proteus spp.</i>	<p>PARTIAL TO COMPLETE INHIBITION</p>  <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 INHIBITED</p>
<i>Pseudomonas aeruginosa</i>	 <p>Growth: +++ Colony: Maroon/red with transparent margin, circular to irregular, raised, entire, 1-2mm</p>	 <p>Growth: +++ Colony: Transparent, circular to slightly irregular, slightly convex, entire to slightly undulate, 0.1-0.2mm (punctiform)</p>
<i>Pseudomonas fluorescens</i>	 <p>Growth: +++ Colony: Clear/colorless with grey/dark center, translucent edges, irregular/spreading to confluent, 2-4mm</p>	 <p>Growth: +++ Colony: Clear to Amber, CVEG, 1-2mm</p>
<i>Salmonella typhimurium</i>	<p>Growth: +++ Colony: Purple/pink, FED, 0.5-1.0mm</p>	<p>INHIBITED</p>

<p><i>Salmonella enteritidis</i></p>	 <p>INHIBITED</p> <p>Growth: +++ Colony: Red, FED, 0.5-1.0mm</p>	
<p><i>Serratia spp.</i></p>	 <p>Growth: ++ Colony: Red, FED, 0.5-1.0mm</p>	 <p>Growth: +++ Colony: Transparent, CVEG, 0.1-0.5mm (punctiform)</p>
<p><i>Shigella spp.</i></p>	 <p>Growth: + Colony: Maroon/red, CVEG, 0.5-1.0mm</p>	 <p>Growth: + Colony: Translucent, irregular, raised, dull, undulate, 1-2mm</p>
<p><i>Staphylococcus spp.</i></p>	 <p>Growth: + Colony: Red, FED, 0.5-1.0mm</p>	 <p>Growth: +++ Colony: Yellow/gold, opaque, CVEG, 0.5-1.0mm</p>
<p><i>Streptococcus spp.</i></p>	 <p>Growth: + Colony: Maroon/red, CVEG, 0.1-0.5mm</p>	 <p>Growth: + Colony: Yellow/gold, opaque, CVEG, 0.5-1.0mm</p>

*Streptomyces
griseus*



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



Growth: +
Colony: White to tan (darker), circular with rough (leathery) center, flat to raised/umbonate, undulate to lobate, 2-5mm+ (to cm)

Gram (+)
Bacteria

PARTIAL TO COMPLETE INHIBITION

GLOSSARY

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