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# NUT/RB

# MICROSLIDE® TECHNICAL DOCUMENT

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# NUT/RB

CODE: M-NUT/RB

## USE

Isolation and differentiation of Gram (-) enteric bacilli. (**NUT**) Selective enumeration and cultivation of yeasts, molds, and Actinomycetes from food and other surfaces (**RB**).

## 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]. Rose Bengal Agar is recommended in *Standard Methods* for the enumeration of yeasts and molds from food and water.

## 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: Rose Bengal Agar (RB)** – (Color: Pink) Selective medium for the enumeration of yeasts and molds.

***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* (RB) 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 $20^2$cm of the surface by contacting the surface twice in separate $10^2$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.

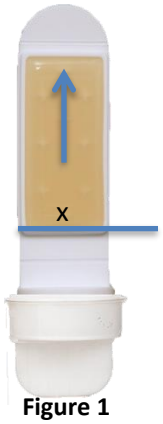



**Figure 1**

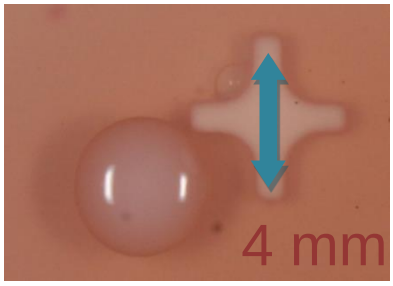
# INCUBATION

| 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.

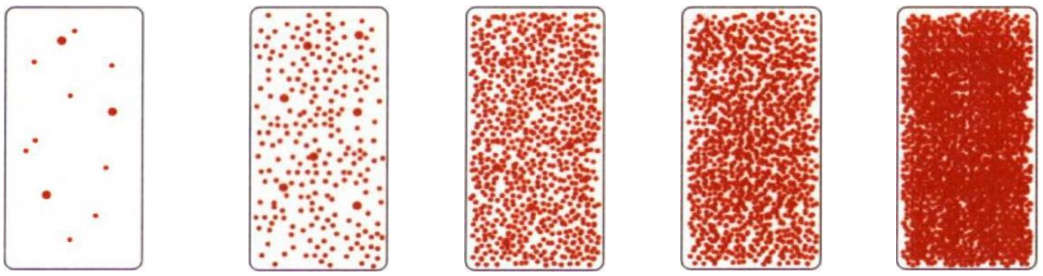
# COLONY MEASURING

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**



**$10^3$ cfu/mL**
(1,000)
**(Light)**

**$10^4$ cfu/mL**
(10,000)

**$10^5$ cfu/mL**
(100,000)
**(Moderate)**

**$10^6$ cfu/mL**
(1,000,000)

**$10^7$ 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.

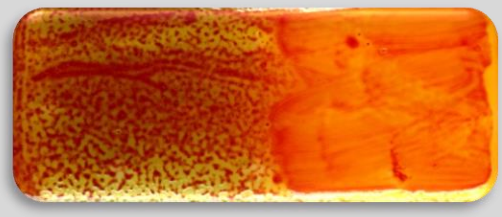
# DISPOSAL

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

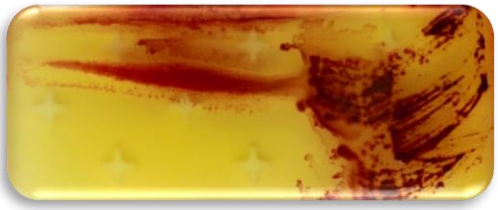
| Organism | Nutrient-TTC (NUT) | Rose Bengal (RB) |
|---|---|---|
| *Actinomyces bovis* | Growth: +<br>Colony: Opaque/tan-grey, CVEG, 1-3mm | Growth: ++<br>Colony: Opaque/tan-grey, CVEG, 1-3mm |
| *Alternaria spp.* | Growth: +<br>Colony: Downy to wooly; flat, grayish, short, aerial hyphae, later becomes greenish black or olive-brown with a light border, 3-9cm | Growth: ++<br>Colony: Suede-like to woolly, initially white to yellow-orange, becoming black to olive-green or grayish, or grayish-green, umbonate with lighter center area, condication (rings), fast-growing, 3-9cm+ (confluent growth) |
| *Aspergillus niger* |  <br>Growth: +++<br>Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++cm |  <br>Growth: +++<br>Colony: Woolly and/or felt-like, forms a carpet, initially white later with jet black fruiting bodies (sporangia), fast-growing (4.5cm in 4 days), 3-9cm+ (confluent growth) |
| *Aspergillus flavus* | Growth: +<br>Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm | Growth: +++<br>Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm+ (confluent growth) |
| *Aspergillus fumigatus* | Growth: +<br>Colony: Granular to cottony, blue-green, green-grey, or green-brown, 3-9cm | Growth: +++<br>Colony: Felt-like, forms a carpet, initialy white to green or blue-green fruiting bodies, 3-9cm+ (confluent growth) |
| *Aspergillus terreus* | Growth: +<br>Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm | Growth: +++ |

| | | |
|---|---|---|
| | | Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm+ (confluent growth) |
| *Bacillus spp.* |  Growth: +++<br>Colony: Green with dark green center |  Growth: ++<br>Colony: Translucent to pink, circular to irregular, flat to raised, entire, 2-5mm |
| *Botrytis spp.* | Growth: +<br>Colony: Wooly, white/grey/brown pigment, 3-9cm | Growth: +++<br>Colony: Wooly, white/grey/brown pigment, 3-9cm |
| *Candida albicans* |  Growth: +++<br>Colony: Cream, CVEG, 1-2mm |  Growth: +++<br>Colony: White to pink, circular, convex, dull, entire, 0.1-0.5mm |
| *Chaetomium spp.* | PARTIAL TO COMPLETE INHIBITION | Growth: +++<br>Colony: Suede-like to Woolly, initially white, later globular (roundish) gray or olive areas / structures (perithecia) looking like cockleburs, 3-5cm+ (confluent growth) |
| *Cladosporium spp.* |  Growth: +<br>Colony: Granular to wooly (velvety), olive-brown to black/brown, sometimes grey on a dark base, 2-5++cm |  Growth: +<br>Colony: Suede-like to woolly, often becoming powdery due to the production of abundant conidia, forms a carpet, white turning olive-brown, buff, or brown, slow-growing, 3-9cm+ (confluent growth) |
| *Epicoccum spp.* | Growth: +<br>Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm | Growth: +++<br>Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm |

| | | |
|---|---|---|
| *E. coli* |  <br>Growth: +++<br>Colony: Yellow/Orange/Red, CVEG, 0.5-1.0mm | INHIBITED |
| *Enterobacter aerogenes* |  <br>Growth: +++<br>Colony: Red, CVEG, 2-4mm | Growth: ++<br>Colony: Pink to red, CVEG, 2-4mm |
| *Enterococcus spp.* | INHIBITED | INHIBITED |
| *Fusarium spp.* |  <br>Growth: +<br>Colony: Wooly, flat, sometimes mucous-like |  <br>Growth: +++<br>Colony: Wooly, initially white, later with yellow, pink, red, purple or pale brown coloring, fast-growing, 3-9cm+ (confluent growth) |
| *Klebsiella spp.* |  <br>Growth: +++<br>Colony: Amber/Red, spreading, 4-5mm | INHIBITED |
| *Microsporum spp.* | Growth: +<br>Colony: Glaborous (smooth), downy, wooly, powdery, white at first, later becoming grayish-yellow to blue-green with age, wrinkled with age, 1-9+cm | Growth: +<br>Colony: Glaborous (smooth), downy, wooly, powdery, white at first, later becoming grayish-yellow to blue-green with age, wrinkled with age, 1-9+cm |

| | | |
|---|---|---|
| *Muccor spp.* |  |  |
| | Growth: +<br>Colony: Wooly, fluffy (like cotton candy), white at first, later becoming gray/yellow to blue-green with age, 2-5++cm | Growth: +<br>Colony: Woolly, initially white, then white-yellow to various shades of gray to green with lollipop fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth) |
| *Penicillium chrysogenum (notatum)* |  |  |
| | Growth: ++<br>Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-5cm | Growth: ++<br>Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-9cm+ (confluent growth) |
| *Penicillium roqueforti* |  |  |
| | Growth: +<br>Colony: Granular, dull, green in coloar, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm | Growth: ++<br>Colony: Granular, velvet-like, flat, initially white then various shades of green, blue-green pigment, 3-9cm+ (confluent growth) |
| *Penicillium digittum* | Growth: +<br>Colony: Wooly, fluffy (like cotton candy), white at first, later becoming green with age, 3-9cm | Growth: +++<br>Colony: Suede-like, woolly, initially white, then various shades of olive green, 3-9cm+ (confluent growth) |
| *Pithomyces spp.* | Growth: +<br>Colony: Powdery, pale/dark grey or brown pigment, 2-9++cm | Growth: +++<br>Colony: Powdery, pale/dark grey or brown to olive green pigment, lighter outer ring with center bullseye, 2-9cm+ (confluent growth) |
| *Proteus spp.* | INHIBITED | INHIBITED |

| | | |
|---|---|---|
| *Pseudomonas aeruginosa* |  <br>Growth: +++<br>Colony: Red, irregular, spreading to confluent, 2-4mm | INHIBITED |
| *Pseudomonas fluorescens* |  <br>Growth: +++<br>Colony: Clear/colorless with grey/dark center, translucent edges, irregular/spreading to confluent, 2-4mm |  <br>Growth: +<br>Colony: Translucent, pinkish, or amber, irregular, raised, undulate, 2-4mm+ |
| *Rhizopus spp.* |  <br>Growth: +++<br>Colony: Cottony, white to black/grey (black fruiting bodies), 2-9++cm |  <br>Growth: +++<br>Colony: Dense, cottony growth, initially white, turning to gray with black fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth) |
| *Saccharomyce cerevisiae* |  <br>Growth: ++<br>Colony: Creamy white to tan, spreading, circular, entire, raised to convex, glistening surface, 5-8mm |  <br>Growth: +++<br>Colony: Translucent to white or cream, CVEG (may be dull), 0.1-0.5mm (punctiform) |
| *Salmonella typhimurium* | Growth: +++<br>Colony: Purple/pink, FED, 0.5-1.0mm | INHIBITED |

| | | |
|---|---|---|
| *Salmonella epidermidis* |  <br>Growth: +<br>Colony: Red, FED, 0.5-1.0mm | INHIBITED |
| *Serratia spp.*<br>*Shigella spp.* | PARTIAL TO COMPLETE INHIBITION<br>  <br>Growth: +<br>Colony: Red, FED, 0.5-1.0mm | INHIBITED<br>INHIBITED |
| *Staphylococcus aureus* |  <br>Growth: +<br>Colony: Red, FED, 0.5-1.0mm | INHIBITED |
| *Streptococcus spp.* |  <br>Growth: +<br>Colony: Red, FED, 0.5-1.0mm | INHIBITED |
| *Torula spp.* |  <br>Growth: +<br>Colony: Arrowhead/circle or heart shape, red, 0.5-1.0mm |  <br>Growth: +++<br>Colony: White, opaque, viscous, CVEG, 0.1-0.5mm (punctiform) |
| *Trichoderma spp.* | Growth: + | Growth: ++ |

| | | |
|---|---|---|
| | Colony: Cottony, white, later scattered green or yellow-green patches (rings), 2-9++cm | Colony: Cottony, white, later scattered green or yellow-green patches (rings), 3-9cm+ (confluent growth) |
| *Trichophyton spp.* | Growth: +<br>Colony: Wooly with indented boarders, white to brown/tan pigment, 2-9++cm | Growth: ++<br>Colony: Wooly , initially white with brownish/tan pigmentation, outer darker ring, 3-9cm+ |
| *Gram (+) Bacteria* | PARTIAL TO COMPLETE INHIBITION | |

# GLOSSARY

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