NUT/RB

CODE: M-NUT/RB

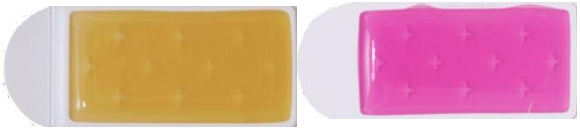
Nutrient-TTC Agar (**NUT**)
Rose Bengal Agar (**RB**)

USE

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

Side 1: Nutrient-TTC Agar (**NUT**) (yellow)
(*Side 1 of each paddle is marked with an indented laser line)

Side 2: Rose Bengal Chloramphenicol Agar (**RB**) (pink)



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 Chloramphenicol Agar is recommended in *Standard Methods* for the enumeration of yeasts and molds from food and water.

PADDLE AGAR

Nutrient-TTC Agar (NUT) – General purpose (relatively non-selective) medium, containing two peptones, which will support the growth of a wide variety of organisms. Suitable for cultivation of both aerobes and anaerobes. This medium contains the dye 2,3,5-triphenyltetrazolium (TTC)[2]. Aerobic coliform bacteria species grow on this medium, and they can be detected by their ability to reduce TTC to a red-colored formozan dye. Bacterial colonies appear as red dots on an otherwise yellow medium. Agar is the solidifying agent. This medium is useful for the recovery of 'stressed coliforms' from chlorinated water. NOTE: Contains the pH indicator bromthymol blue. 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.

Rose Bengal Chloramphenicol Agar (RB) – Selective medium for the enumeration of fungi. This formula is prepared with a neutral pH and supplemented with chloramphenicol as the selective agent in fungal medium. Rose Bengal Chloramphenicol Agar is recommended in standard methods for the enumeration of yeast and molds from food and water. It is also referred to as Rose Bengal Agar and Rose Bengal-Malt Extract Agar. Agar and a proprietary polymer are the solidifying agents.

[1] United States Pharmacopeial Convention. 2007. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.
[2] Chapman, G.H> 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. 53:504.

CULTURE CONTROLS

10-300 inoculum (CFU)

	NUT Agar	RB Agar
Aspergillus niger	INHIBITED	GROWTH
Enterococcus faecalis	GROWTH	INHIBITED
Esherichia coli	GROWTH	INHIBITED
Proteus mirabilis	INHIBITED	INHIBITED
Salmonella typhimurium	GROWTH	INHIBITED

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 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.

SAMPLING

Detection Limit: TPC > 100cfu/mL
Paddle surface area: 12.5 sq cm (2.5 x 5cm)

Direct Contact / Spread Sampling provides for the rapid monitoring of total colony count (TCC) of surfaces, liquids and solid materials.

SURFACE Sampling Protocol

1. Remove the paddle from the vial. Do not touch the agar surfaces. Use aseptic techniques.
2. Firmly press the paddles (2X contact) against the test surface for a minimum of 3-5 seconds (15 seconds, optimal) for a 1:1 contact transfer. (See Notes below)
3. Replace paddle in vial.
4. Incubate @ 25-35°C ± 2°C for 18-24 hours.

Notes:

- Microslides® are similar to RODAC[3] plates. The literature reports a 41% (aerosolized *Bacillus subtilis*) spores from stainless steel surfaces (47% swab vs. 41% RODAC). Results from the RODAC recovery method are more reproducible than those of the swab technique[4] [5].
- A 50% recovery rate is "usual" and Microslide® users should double-contact a surface to achieve a 1:1 contact transfer.

LIQUID Sampling Protocol

DIRECT IMMERSION PROTOCOL – low viscous liquids

[3] RODAC – Replicate Organism Detection and Counting
[4] Angelotti, R; Wilson, J.L.; Litsky, W; Walter, W.G. Comparative evaluation of the cotton swab and RODAC methods for the recovery of *Bacillus subtilis* spore contamination from stainless steel surfaces. Health lab Sci. 1:289-296; 1964.
[5] Buggy, B. et al. 1983. Comparison of Methods for Recovery of Clostridium Difficile From an Environmental Surface. J Clin Microbiol. 18(2):348-352.

1. Mix liquid test sample.
2. Remove the paddle from the vial. Follow aseptic technique; 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[6] (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 @ 25-35°C ± 2°C for 18-24 hours.

SPREAD Protocol – high viscous liquids or precise inoculation volumes

1. Mix liquid test sample.
2. Using aseptic technique, remove paddle from vial. Do not touch the agar surfaces.
3. Holding the contact agar surface on a horizontal plane, pipet 330µL[7] (0.33mL) (deposit volume as a single drop (X)) approximately 1cm from the handle boundary (Figure 1).
4. Position a sterile glass rod on the "handle" side of the drop (x) 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 @ 25-35°C ± 2°C for 18-24 hours.

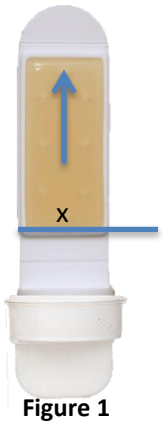


Figure 1

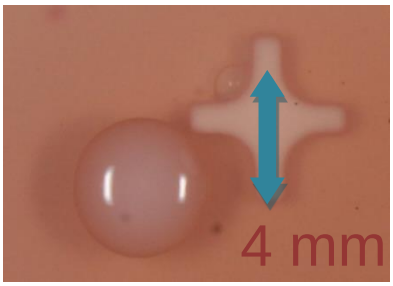
INCUBATION

Incubate @ 25-35°C ± 2°C for 24-48 hours. Enumerate. Incubation after 48 hours may produce confluent growth making enumeration more difficult.

TEMPERATURE	MINIMUM INCUBATION PERIOD	OPTIMAL INCUBATION PERIOD
20-25°C (fungi)	72 hours	5-7 days
35°C (bacteria)	5 days	7 days

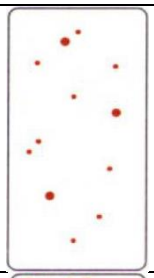
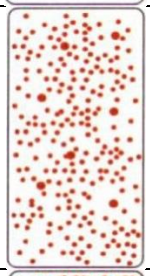
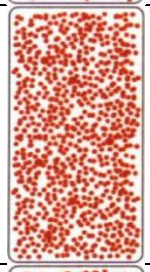
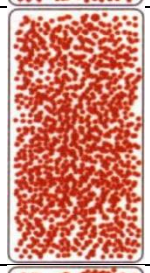
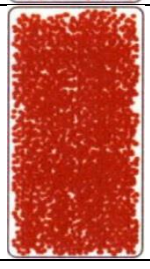
COLONY MEASURING

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.



[6] Retention of bacteria in liquid films on agar surfaces after immersion in bacterial suspensions is a simple dilution effect: the number retained is dependent only upon suspension population density. C.J. Thomas et. al. Retention of Bacteria in Liquid Films at agar surfaces. *Applied and Environmental Microbiology*. Vol. 34; No. 4, p. 456-457, 1977.
[7] Typically, this volume is 0.1mL (100µL). A higher volume is used to accommodate the "bread loaf" topography of the paddle agar surface.

ENUMERATION

Total Colony Count (TCC)	Enumeration Panel Pictogram	Surface	Liquid
0		<1 cfu/cm^2	<100 cfu/mL
1-5		<1 cfu/cm^2	100 cfu/mL
10-50		1 cfu/cm^2	10^3 cfu/mL
100-500		10 cfu/cm^2	10^4 cfu/mL
>500		45 cfu/cm^2	10^5 cfu/mL
>1,000 (partial confluency)		80 cfu/cm^2	10^6 cfu/mL
>10,000 (confluency TNTC)		>100 cfu/cm^2	10^7 cfu/mL

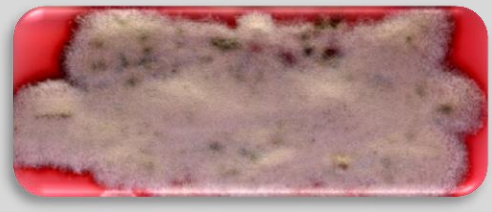
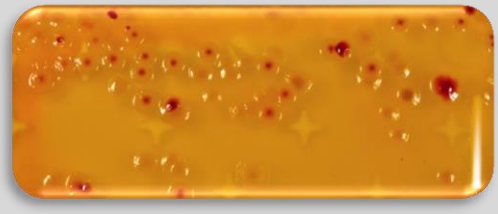
EXAMPLE:
Nutrient-TTC
Inoculated paddle showing approximately 1000 CFU / 100mL.

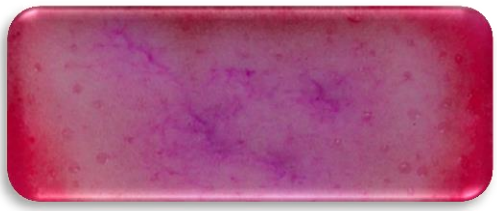
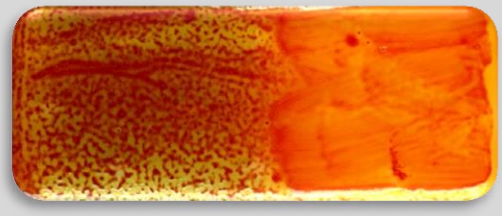
DISPOSAL

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.

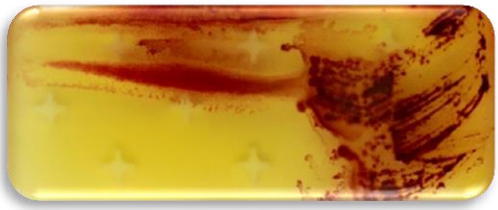
IDENTIFICATION

Organism	Nutrient-TTC (NUT)	Rose Bengal (RB)
Actinomyces bovis	Growth: + Colony: Opaque/tan-grey, CVEG, 1-3mm	Growth: ++ Colony: Opaque/tan-grey, CVEG, 1-3mm
Alternaria spp.	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
Aspergillus niger	 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, 3-9cm
Aspergillus flavus	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
Aspergillus fumagatus	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
Aspergillus	Growth: +	Growth: +++

terreus	Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm	Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm
Bacillus spp.	 Growth: +++ Colony: Green with dark green center	 Growth: + Colony: Pink, 0.5-1.0mm
Botrytis spp.	Growth: + Colony: Wooly, white/grey/brown pigment, 3-9cm	Growth: +++ Colony: Wooly, white/grey/brown pigment, 3-9cm
Candida albicans	 Growth: +++ Colony: Cream, CVEG, 1-2mm	 Growth: +++ Colony: Pink, spreading, 6mm
Chaetomium spp.	PARTIAL TO COMPLETE INHIBITION	Growth: +++ Colony: Wooly, white/grey/olive, 3-5cm
Cladosporium spp.	 Growth: + Colony: Granular to wooly (velvety), olive-brown to black/brown, sometimes grey on a dark base, 2-5++cm	 Growth: + Colony: Granular to wooly (velvety), white turning olive-brown to black, sometimes grey on a dark base, 3-9cm
Epicoccum spp.	Growth: + Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm	Growth: +++ Colony: Wooly, cottony, felty, yellow/orange/red, 3-5cm
E. coli	 Growth: +++ Colony: Yellow/Orange/Red, CVEG, 0.5-	INHIBITED

	1.0mm	
Enterobacter aerogenes	 Growth: +++ Colony: Red, CVEG, 2-4mm	Growth: ++ Colony: Pink to red, CVEG, 2-4mm
Enterococcus spp.	INHIBITED	INHIBITED
Fusarium spp.	 Growth: + Colony: Wooly, flat, sometimes mucous-like	 Growth: +++ Colony: Wooly, flat (sometimes mucous-like), white, yellow, pink, purple, or pale brown, 3-9cm
Klebsiella spp.	 Growth: +++ Colony: Amber/Red, spreading, 4-5mm	INHIBITED
Microsporum spp.	Growth: + 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: + 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: + Colony: Wooly, fluffy (like cotton candy), white at first, later becoming gray/yellow to blue-green with age, 2-5++cm	 Growth: + Colony: Wooly, velvety, with regular margins, white at first, becoming grayish/blue-green with age, 3-9cm

Penicillium chrysogenum (notatum)	 Growth: ++ Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-5cm	 Growth: ++ Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-5cm
Penicillium roqueforti	 Growth: + Colony: Granular, dull, green in coloar, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm	 Growth: ++ Colony: Granular, dull, green in coloar, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm
Penicillium digittum	Growth: + Colony: Wooly, fluffy (like cotton candy), white at first, later becoming green with age, 3-9cm	Growth: +++ Colony: Wooly, fluffy (like cotton candy), white at first, later becoming green with age, 3-9cm
Pithomyces spp.	Growth: + Colony: Powdery, pale/dark grey or brown pigment, 2-9++cm	Growth: +++ Colony: Powdery, pale/dark grey or brown pigment, 2-9++cm
Proteus spp.	INHIBITED	INHIBITED
Pseudomonas aeruginosa	 Growth: +++ Colony: Red, irregular, spreading to confluent, 2-4mm	INHIBITED
Pseudomonas fluorescens	 Growth: +++	 Growth: +

	Colony: Clear/colorless with grey/dark center, translucent edges, irregular/spreading to confluent, 2-4mm	Colony: Red/pink, irregular, spreading to confluent, 2-4mm
Rhizopus spp.		
	Growth: +++ Colony: Cottony, white to black/grey (black fruiting bodies), 2-9++cm	Growth: +++ Colony: Cottony, white to black-grey (black fruiting bodies), 3-9cm
Saccharomyce cerevisiae		
	Growth: ++ Colony: Creamy white to tan, spreading, circular, entire, raised to convex, glistening surface, 5-8mm	Growth: +++ Colony: Pink, FED (maybe glossy), 1-3mm
Salmonella typhimurium	Growth: +++ Colony: Purple/pink, FED, 0.5-1.0mm	INHIBITED
Salmonella epidermidis		INHIBITED
	Growth: + Colony: Red, FED, 0.5-1.0mm	
Serratia spp.	PARTIAL TO COMPLETE INHIBITION	INHIBITED
Shigella spp.		INHIBITED
	Growth: + Colony: Red, FED, 0.5-1.0mm	

Staphylococcus aureus	 Growth: + Colony: Red, FED, 0.5-1.0mm	INHIBITED
Streptococcus spp.	 Growth: + Colony: Red, FED, 0.5-1.0mm	INHIBITED
Torula spp.	 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
Trichoderma spp.	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
Trichophyton spp.	Growth: + Colony: Wooly with indented boarders, white to brown/tan pigment, 2-9++cm	Growth: + Colony: Wooly with indented boarders, white to brown/tan pigment, 2-9++cm
Gram (+) Bacteria	PARTIAL TO COMPLETE INHIBITION	

GLOSSARY

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