MAKINGCOSMETICS

TSA/RB

CODE: M-TSA/RB

Trypticase Soy Agar (**TSA**)
Rose Bengal Agar (**RB**)

USE

Cultivation of a wide varietiy of aerobic and anaerobic microorganisms[1] (**TSA**). Selective enumeration and cultivation of yeasts, molds, and Actinomycetes from food and other surfaces (**RB**).

Side 1: Trypticase Soy Agar (**TSA**) (off-white, opaque)
(*Side 1 of each paddle is marked with an indented laser line)

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

APPLICATION

TSA is recommended in multiple water and wastewater applications[2], and numerous standard methods for food testing[3]. TSA is commonly used as a maintenance medium for culture collections, and testing bacterial contaminants in cosmetics[4]. Rose Bengal Chloramphenicol Agar is recommended in *Standard Methods* for the enumeration of yeasts and molds from food and water.

PADDLE AGAR

Trypticase Soy Agar (TSA) – In 1955, Leavitt et al.[5] discovered Tryptic Soy Agar facilitated vigorous growth of aerobic and anaerobic microorganisms. This is an enriched media, suitable to support fastidious heterotrophs.

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.

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[1] United States Pharmacopeial Convention. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial convention, Rockville, MD.
[2] Greenberg, A.E., L.S. Clesceri, and A.D. Eaton (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
[3] U.S. Food and Drug Administration. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
[4] Curry, A.S., G.G. Joyce, and G.N. McEwen, Jr. 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc. Washington, D.C
[5] Leavitt, J.M., I.J., Naidorf and P. Shugaevsky. 1955. The undetected anaerobe in endodontics: a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist. 25:377-382.

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CULTURE CONTROLS

10-300 inoculum (CFU)

	TSA Agar	RB Agar
Aspergillus niger	GROWTH	GROWTH
Bacillus subtilis	GROWTH	INHIBITED
Candida albicans	GROWTH	INHIBITED
Escherichia coli	GROWTH	INHIBITED
Pseudomonas aeruginosa	GROWTH	INHIBITED
Staphylococcus aureus	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 @ 25-30°C ± 2°C for 18-24 hours.

Notes:

- Microslides® are similar to RODAC[6] 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[7] [8].
- A 50% recovery rate is "usual" and Microslide® users should double-contact a surface to achieve a 1:1 contact transfer.

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[6] RODAC – Replicate Organism Detection and Counting

[7] 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.

[8] Buggy, B. et al. 1983. Comparison of Methods for Recovery of Clostridium Difficile From an Environmental Surface. J Clin Microbiol. 18(2):348-352.

LIQUID Sampling Protocol

DIRECT IMMERSION PROTOCOL – low viscous liquids

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[9] (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 @ 25-30°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[10] (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 @ 25-30°C ± 2°C for 18-24 hours.

Figure 1

INCUBATION

Incubate @ 25-30°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
25°C (fungi)	72 hours	5-7 days
30°C (bacteria)	5 days	7 days

[9] 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.

[10] Typically, this volume is 0.1mL (100µL). A higher volume is used to accommodate the "bread loaf" topography of the paddle agar surface.

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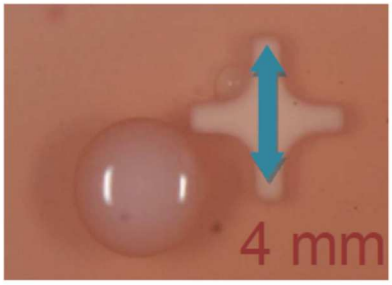
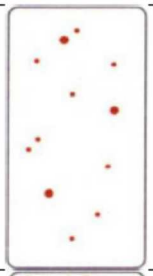
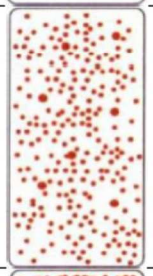
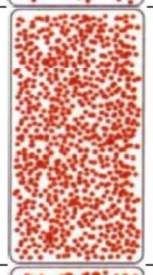
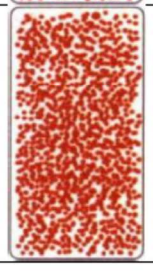
COLONY MEASURING

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

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

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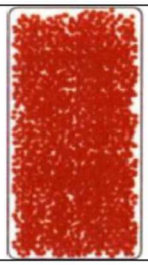
>10,000 (confluency TNTC)		>100 cfu/cm^2	10^7 cfu/mL

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	Tryptic Soy (TSA)	Rose Bengal (RB)
Actinomyces bovis	PARTIAL TO COMPLETE INHIBITION	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, white with jet black fruiting bodies, yellow/grey hyphae, 3-9cm	 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 terreus	Growth: ++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm	Growth: +++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm

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Bacillus spp.	 Growth: ++ Colony: Translucent/Cream, 0.5-1.0mm	 Growth: + Colony: Pink, 0.5-1.0mm
Botrytis spp.	Growth: ++ Colony: Wooly, white/grey/brown, 3-9cm	Growth: +++ Colony: Wooly, white/grey/brown, 3-9cm pigment, 3-9cm
Candida albicans	 Growth: +++ Colony: Cream, CVEG, 1-2mm	 Growth: +++ Colony: Pink, spreading, 6mm
Chaetomium spp.	Growth: ++ Colony: Wooly, white/grey/olive, 3-5cm	Growth: +++ Colony: Wooly, white/grey/olive, 3-5cm
Cladosporium spp.	 Growth: + Colony: Granular to wooly (velvety), white turning olive-brown to black, sometimes grey on a dark base, 3-9cm	 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, CVEG, 0.5-1.0mm	INHIBITED
Enterobacter	Growth: +++	Growth: ++

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aerogenes *Fusarium spp.*	Colony: Clear to amber, CVEG, 2-4mm	Colony: Pink to red, CVEG, 2-4mm
		
	Growth: +++ Colony: Wooly, flat (sometimes mucous-like), white, yellow, pink, purple, or pale brown, 3-9cm	Growth: +++ Colony: Wooly, flat (sometimes mucous-like), white, yellow, pink, purple, or pale brown, 3-9cm
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, velvety, with regular margins, white at first, becoming grayish/blue-green with age, 3-9cm	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
Penicilium roqueforti		

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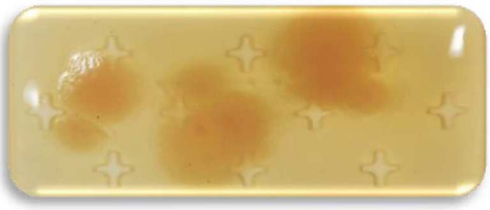
	Growth: ++ Colony: Granular, dull, green in coloar, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm	Growth: ++ Colony: Granular, dull, green in coloar, arachnoid (with many spider web-like fibers) colony margins, 0.5-1.0cm
Penicillium digittum	Growth: +++ Colony: Wooly, fluffy (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
Pseudomonas aeruginosa	 Growth: +++ Colony: Colorless with dark centers, translucent edges, irregular, spreading to confluent, diffusible green-blue pigment, 2-4mm	INHIBITED
Pseudomonas fluorescens	 Growth: +++ Colony: Colorless/Yellow with dark center, irregular, spreading to confluent, 2-4mm	 Growth: + Colony: Red/pink, irregular, spreading to confluent, 2-4mm
Rhizous spp.	 Growth: +++ Colony: Cottony, white to black-grey (black fruiting bodies), 3-9cm	 Growth: +++ Colony: Cottony, white to black-grey (black fruiting bodies), 3-9cm

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Saccharomyces cerevisiae	 Growth: +++ Colony: Colorless, FED (maybe glossy), 1-3mm	 Growth: +++ Colony: Pink, FED (maybe glossy), 1-3mm
Salmonella typhimurium	Growth: +++ Colony: Purple/pink, Fed, 0.5-1.0mm	INHIBITED
Salmonella epidermidis	 Growth: + Colony: Clear/translucent, FED, 0.5-1.0mm	INHIBITED
Stachybotrys spp.	Growth: + Colony: Powdery, white, pink, orange, or black, 3-9cm	Growth: ++ Colony: Powdery, white, pink, orange, or black, 3-9cm
Torula spp.	 Growth: + Colony: Arrowhead/circle or heart shape, grey/white to brown with age, 3-9cm	 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

GLOSSARY

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

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