

TSA/RB

Code 5552

COMING SOON! 

BioPaddles™ Colony Identification App

Tryptic Soy Agar (**TSA**)
Rose Bengal Chloramphenicol (**RB**)

USE:

Cultivation of a wide variety of aerobic and anaerobic microorganisms¹. (**TSA**)
Selective enumeration and cultivation of yeasts, molds and Actinomycetes from food and other surfaces. (**RB**)

Side 1: Tryptic 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

Tryptic Soy Agar is recommended in multiple water & wastewater applications², 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 Examination of Water and Wastewater for the enumeration of yeasts and molds from foods and water.

PADDLE AGARS

Tryptic Soy Agar (TSA) – In 1955, Leavitt et al.⁵ discovered that 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. RB Agar 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. 1995. The United States Pharmacopeia, 23rd ed. The United State 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.

CULTURE CONTROLS

10-100 inoculum (CFU)

	TSA Agar	EMB Agar
<i>Aspergillus niger</i>	GROWTH	GROWTH
<i>Bacillus subtilis</i>	GROWTH	INHIBITED
<i>Candida albicans</i>	GROWTH	INHIBITED
<i>Escherichia coli</i>	GROWTH	INHIBITED
<i>Pseudomonas aeruginosa</i>	GROWTH	INHIBITED
<i>Staphylococcus aureus</i>	GROWTH	INHIBITED

STORAGE / EXPIRATION

Store tightly sealed BioPaddles™ in a cool, dry location (less than 68°F/20°C). Avoid temperature changes. BioPaddles™ may be refrigerated, but it is not necessary. Do not freeze. If freezing occurs, thaw (3-6 hours) under refrigeration temperatures (40°F; 4.4°C). Freezing can promote excess water loss and variation in media surface due to crystal formation. The average shelf-life is one year. Refer to expiration date (See: BBE stamped on vial). Discard if paddle agar appears oxidized (darkens from expected color). The expiration date applies to the medium in an intact container when stored as directed.

SAMPLING

Liquids: Twist to remove paddle from vial. Fill vial to 40 mL fill line with the liquid to be sampled. The 40 mL volume can be used to calculate Total Viable Count (TVC) and/or Total Colony Count (TCC). Replace paddle. Allow a contact time of 15 seconds. Remove the paddle. Empty the vial. Replace the paddle in the vial.



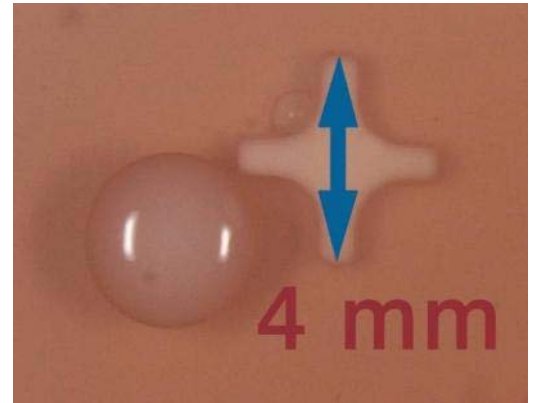
Surfaces: Twist to remove paddle from vial. Allow the paddle surface (10 cm²) to come into physical contact with the test surface. Recovery rate is about 50%. To insure an accurate recovery, gently sweep (or touch) the paddle to cover a 20 cm² area. Replace paddle in vial.

INCUBATION






Incubate at 30 - 35°C for 24-48 hours. Growth will be present on the TSA agar, as it is enriched and non-selective. Growth may or may not be present on the Rose Bengal agar after this brief period as it is selective for yeasts and molds which tend to like cooler temperatures and grow more slowly in general. For this reason, re-incubate and check again after 2 to 4 days.

COLONY MEASURING


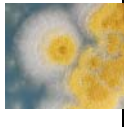


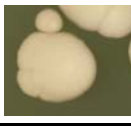





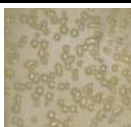
Each BioPaddles™ paddle has molded media attachment points that are 4mm in length (point-to-point). This feature provides a useful guidepost to estimating nearby colony size.









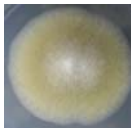




IDENTIFICATION

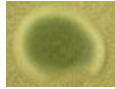
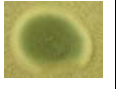
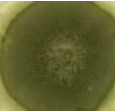
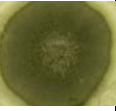






ORGANISM		TSA			RB		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE
<i>Actinomyces bovis</i>	<ul style="list-style-type: none"> • Lactose (+ / v) • Indole (v) ◆ • Oxidase (-) ◆ • Catalase (-) ◆ • Urease (-) ◆ • Gram (+) Rod 	PARTIAL - COMPLETE INHIBITION	---	---	++	<ul style="list-style-type: none"> • Opaque / tan-gray • CVEG • 1-3mm 	
<i>Alternaria spp.</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	+	<ul style="list-style-type: none"> • Downy to woolly; flat • Grayish, short, aerial hyphae • later becomes greenish black or olive brown with a light border • 3-9cm 		++	<ul style="list-style-type: none"> • Downy to woolly; flat • Grayish, short, aerial hyphae • later becomes greenish black or olive brown with a light border • 3-9cm 	
<i>Aspergillus niger</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	+	<ul style="list-style-type: none"> • Granular • Jet black conidia w/ yellow/gray hyphae • 3-9cm 		+++	<ul style="list-style-type: none"> • Granular • White, w/ jet black fruiting bodies w/ yellow/gray hyphae • 3-9cm 	

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ORGANISM		TSA			RB		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE
<i>Aspergillus flavus</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Granular to wooly Yellow, yellow-green or yellow-brown pigment 3-9cm 		+++	<ul style="list-style-type: none"> Granular to wooly Yellow, yellow-green or yellow-brown pigment 3-9cm 	
<i>Aspergillus fumigatus</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Granular to cottony Blue-green, green-gray, green-brown pigment 3-9cm 		+++	<ul style="list-style-type: none"> Granular to cottony Blue-green, green-gray, green-brown pigment 3-9cm 	IMAGE PENDING
<i>Aspergillus terreus</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Granular, radially rugose (wrinkled) Cinnamon buff, brown pigment 3-9cm 	IMAGE PENDING	+++	<ul style="list-style-type: none"> Granular, radially rugose (wrinkled) Cinnamon buff, brown pigment 3-9cm 	IMAGE PENDING
<i>Botrytis spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Wooly white, grey/brown pigment 3-9cm 		+++	<ul style="list-style-type: none"> Wooly white, grey/brown pigment 3-9cm 	IMAGE PENDING
<i>Candida albicans</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+++	<ul style="list-style-type: none"> Cream CVEG 1-2mm 		+++	<ul style="list-style-type: none"> Pink Spreading 6mm 	
<i>Chaetomium spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	++	<ul style="list-style-type: none"> Wooly white, grey/olive pigment 3-5cm 		+++	<ul style="list-style-type: none"> Wooly white, grey/olive pigment 3-5cm 	
<i>Cladosporium spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Granular to wooly (velvety) Olive-brown to blackish-brown (sometimes gray) on a dark base 3-9cm 		+	<ul style="list-style-type: none"> Granular to wooly (velvety) Olive-brown to blackish-brown (sometimes gray) on a dark base 3-9cm 	
<i>Epicoccum spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+++	<ul style="list-style-type: none"> Wooly / Cottony / Felty yellow, orange, red, brown pigment 3-5cm 	IMAGE PENDING	+++	<ul style="list-style-type: none"> Wooly / Cottony / Felty yellow, orange, red, brown pigment 3-5cm 	IMAGE PENDING
<i>E. coli</i>	<ul style="list-style-type: none"> Lactose (+) Indole (+) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod 	+++	<ul style="list-style-type: none"> Yellow / Orange CVEG 0.5 - 1.0mm 		INHIBITED	---	---

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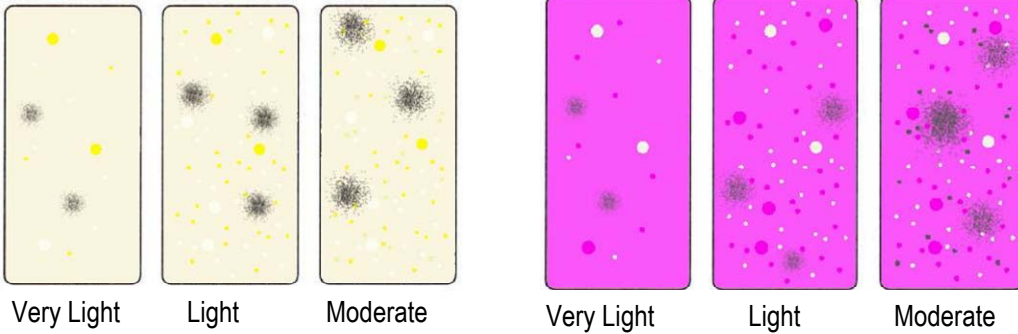
ORGANISM		TSA			RB		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE
<i>Enterobacter aerogenes</i>	<ul style="list-style-type: none"> Lactose (+) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod 	+++	<ul style="list-style-type: none"> Clear to amber CVEG 2-4mm 		++	<ul style="list-style-type: none"> Pink to Red CVEG 2-4mm 	
<i>Fusarium spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+++	<ul style="list-style-type: none"> Woolly / Flat (sometimes mucous-like) White, yellow, pink, purple, or pale brown pigment 3-9cm 		+++	<ul style="list-style-type: none"> Woolly / Flat (sometimes mucous-like) White, yellow, pink, purple, or pale brown pigment 3-9cm 	
<i>Microsporium spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+	<ul style="list-style-type: none"> Glabrous (smooth) / Downy / Woolly / Powdery White at first, later becoming grayish yellow to blue green with age Wrinkled w/ age 1-9cm 		+	<ul style="list-style-type: none"> Glabrous (smooth) / Downy / Woolly / Powdery White at first, later becoming grayish yellow to blue green with age Wrinkled w/ age 1-9cm 	
<i>Mucor spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Zygomycete 	+	<ul style="list-style-type: none"> Woolly, velvety, with regular margins White at first, later becoming grayish yellow to blue green with age 3-9cm 		+	<ul style="list-style-type: none"> Woolly, velvety, with regular margins White at first, later becoming grayish yellow to blue green with age 3-9cm 	
<i>Penicillium chrysogenum (notatum)</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	++	<ul style="list-style-type: none"> Granular, velvet-like/powdery, flat Initially white, then various shades of green blue-green or yellow-green pigment 3-5cm 		++	<ul style="list-style-type: none"> Granular, velvet-like/powdery, flat Initially white, then various shades of green blue-green or yellow-green pigment 3-5cm 	
<i>Penicillium roqueforti</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	++	<ul style="list-style-type: none"> Granular, dull green in color arachnoid (with many spider-web-like fibers) colony margins) 0.5-1cm 		++	<ul style="list-style-type: none"> Granular, dull green in color arachnoid (with many spider-web-like fibers) colony margins) 0.5-1cm 	IMAGE PENDING

ORGANISM		TSA			RB		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE
<i>Penicillium digittum</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+++	<ul style="list-style-type: none"> Woolly, Fluffy (like cotton candy) White at first, later becoming green with age 3-9cm 		+++	<ul style="list-style-type: none"> Woolly, Fluffy (like cotton candy) White at first, later becoming green with age 3-9cm 	
<i>Trichomyces spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	++	<ul style="list-style-type: none"> Powdery Pale/dark grey or brown pigment 3-9cm 		+++	<ul style="list-style-type: none"> Powdery Pale/dark grey or brown pigment 3-9cm 	
<i>Pseudomonas aeruginosa</i>	<ul style="list-style-type: none"> Lactose (-) Indole (-) ◆ Oxidase (+) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod Fluoresces blue under long-wave UV light (400-nm) 	+++	<ul style="list-style-type: none"> Colorless w/ dark centers; translucent edges Irregular; Spreading to confluent Diffusible green-blue pigment 2-4mm 	IMAGE PENDING	INHIBITED	---	---
<i>Pseudomonas fluorescens</i>	<ul style="list-style-type: none"> Lactose (-) Indole (-) ◆ Oxidase (+) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod Fluoresces blue-green under long-wave UV light (400-nm) 	+++	<ul style="list-style-type: none"> Clear or colorless Irregular; Spreading to confluent Clear to grayish with dark centers (translucent edges) 2-4mm 		+/-	<ul style="list-style-type: none"> Red, pink Irregular; Spreading to confluent 2-4mm 	
<i>Rhizopus spp.</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Zygomycete 	+++	<ul style="list-style-type: none"> Cottony white to blackish grey (black fruiting bodies) 3-9cm 		+++	<ul style="list-style-type: none"> Cottony white to blackish grey (black fruiting bodies) 3-9cm 	
<i>Saccharomyces cerevisiae</i>	<ul style="list-style-type: none"> Catalase (+) ◆ Ascomycete 	+++	<ul style="list-style-type: none"> Pink FED (maybe glossy) 1-3mm 		+++	<ul style="list-style-type: none"> Pink FED (maybe glossy) 1-3mm 	
<i>Salmonella typhimurium</i>	<ul style="list-style-type: none"> Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod 	+++	<ul style="list-style-type: none"> Purple / Pink FED 0.5 - 1.0mm 	IMAGE PENDING	INHIBITED	---	---

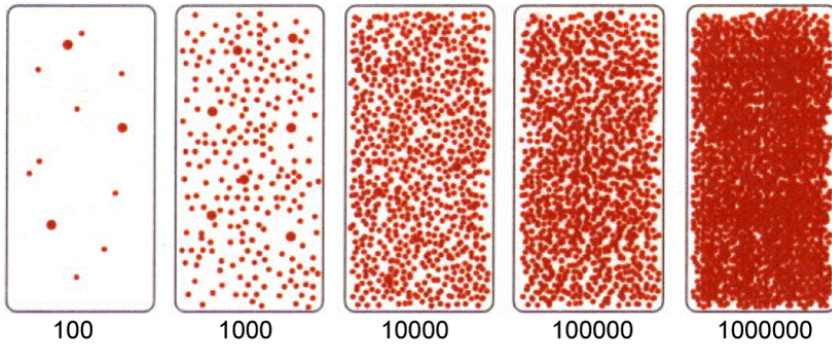
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ORGANISM		TSA			RB		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE
<i>Salmonella epidermidis</i>	<ul style="list-style-type: none"> • Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod 	+	<ul style="list-style-type: none"> • Red • FED • 0.5 - 1.0mm 	IMAGE PENDING	INHIBITED	---	---
<i>Stachybotrys spp.</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	+	<ul style="list-style-type: none"> • Powdery • white, pink, orange, black • 3-9cm 		++	<ul style="list-style-type: none"> • Powdery • white, pink, orange, black • 3-9cm 	IMAGE PENDING
<i>Torula spp.</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	+	<ul style="list-style-type: none"> • Arrowhead / Circle or Heart shape • Grey, white to brown pigment with age • 3-9cm 	IMAGE PENDING	+	<ul style="list-style-type: none"> • Arrowhead / Circle or Heart shape • Grey, white to brown pigment with age • 3-9cm 	IMAGE PENDING
<i>Trichoderma spp.</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	++	<ul style="list-style-type: none"> • Cottony • White / later scattered green or yellow-green patches (rings) • 3-9cm 		++	<ul style="list-style-type: none"> • Cottony • White / later scattered green or yellow-green patches (rings) • 3-9cm 	
<i>Trichophyton spp.</i>	<ul style="list-style-type: none"> • Catalase (+) ◆ • Ascomycete 	+	<ul style="list-style-type: none"> • Woolly with indented borders • White to brownish-tan pigment • 3-9cm 		+	<ul style="list-style-type: none"> • Woolly with indented borders • White to brownish-tan pigment • 3-9cm 	

ENUMERATION



TVC / TCC



Approximate Colony Count per 100 mL

**TVC/TCC
(Total Viable Count/
Total Colony Counts)**

Colony Counts < 1000
Count colonies
TVC/TCC Count = Count x 2.5

Colony Counts > 1000
Use chart
TVC/TCC Count = Count x 2.5

(Based on a 40 mL sample)



Example:
Inoculated NUT/TTC
paddle showing
approximately 1000
CFU/100 mL.

DISPOSAL

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

GLOSSARY:

CVEG	Convex, Entire, Glossy
FED	Full, Entire, Dull
Catalase	Cat (+) contains catalase enzymes that degrade cellular H ₂ O ₂ .
Lactose	Lac (+) bacteria can ferment available lactose in the medium producing an acid which lowers the pH. Lac (-) are non-fermenting.
Indole	Biochemical test to determine the ability of an organism to split indole from the amino acid tryptophan. <i>P. vulgaris</i> is indole (+) while <i>P. mirabilis</i> is indole (-).
Oxidase	OX (+) contains cytochrome c oxidase. In contact with an indicator turns dark blue if OX (+); colorless if OX(-).
Urease	UR (+) presence of enzyme urease which hydrolyzes urea into CO ₂ & NH ₄ .
β-D-Glucuronidase	Glu (+) Found in 97% of <i>E. coli</i> strains. The presence of <i>E. coli</i> is determined when both β-D-Glucuronidase and Indole are (+), and the organism is Gram (-).
Gram	Gram reaction