LaMotte BioPaddles® TECH DOCUMENT
Call: 800-344-3100
Email: tech@lamotte.com

TSA/RB

Code 5552

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

USE:

Cultivation of a wide variety of aerobic and anaerobic microorganisms[1]. (**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 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 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, 8thed., 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.

LaMotte_BioPaddles_TSA_RB 07.22

CULTURE CONTROLS

10-100 inoculum (CFU)

	TSA Agar	RB Agar
Aspergillus niger	GROWTH	GROWTH
Bacilus subtilis	GROWTH	INHIBITED
Candida albicans	GROWTH	INHIBITED
Escherichia coli	GROWTH	INHIBITED
Pseudomonas aeruginosa	GROWTH	INHIBITED
Staphylococcus aureus	GROWTH	INHIBITED

STORAGE / EXPIRATION

Store tightly sealed BioPaddles® in a cool, dry location. Shield from direct sunlight. Store BioPaddles® at room temperature (65 - 77°F/18 - 25°C). Avoid sudden temperature changes. Temperature fluctuations may result in condensation settling at the bottom of the vial. This will not affect the culture properties but could reduce the shelf-life or cause the agar to separate from the plastic paddle support. Do not refrigerate or store at temperatures above 80°F/27°C. Refrigeration may result in water condensation. Avoid freezing. Freezing can promote excess water loss and variation in media surface due to crystal formation. If freezing occurs, wrap BioPaddle in vial in thick towel and thaw at room temperature for 3-6 hours.

Refer to Best Before End date (See: BBE stamped on vial). Discard if paddle agar appears oxidized and darker than the expected color or if contaminants appear. The expiration date is based on medium in an intact container that is 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: Recovery rate is about 50%. Twist paddle to remove from vial. To ensure an accurate recovery, touch the paddle surface (10 cm^2) to the test surface twice to cover a 20 cm^2 area (2 X 10 cm^2). Allow 15 second contact time. Replace paddle in vial.

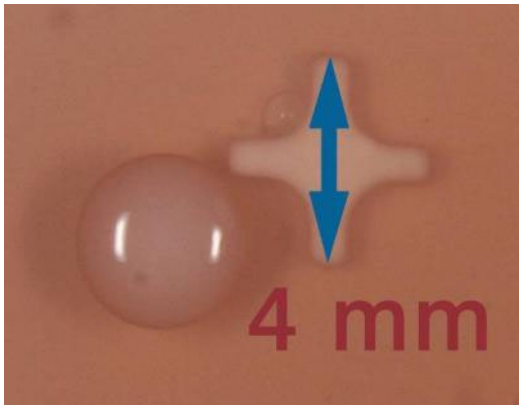
INCUBATION

Temperature	Minimum Period	Optimal Period
35°C	18 hours	24 hours
20-25°C	5 days	7 days

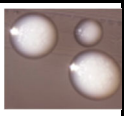
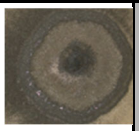
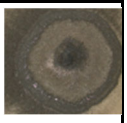
Incubate at 30 - 35°C for 18 hours. Growth will be present on the TSA agar, as it is enriched and non-selective. Growth may or may not be present on the Rose Bengal agar after this brief period as it is selective for yeasts and molds which tend to like cooler temperatures and grow more slowly in general. For this reason, re-incubate and check again after 2 to 4 days.

COLONY MEASURING

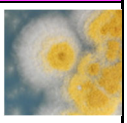
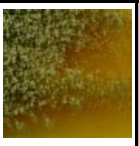
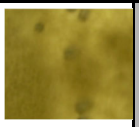
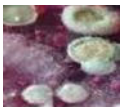
Each BioPaddles® paddle has molded media attachment points that are 4mm in length (point-to-point). This feature provides a useful guidepost to estimating nearby colony size.

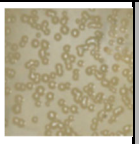


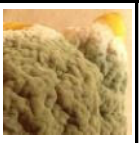
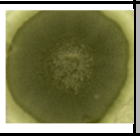
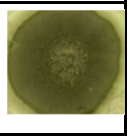
IDENTIFICATION

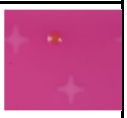
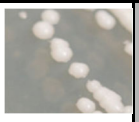
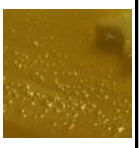
ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Actinomyces bovis	• Lactose (+ / v) • Indole (v) ◆ • Oxidase (-) ◆ • Catalase (-) ◆ • Urease (-) ◆ • Gram (+) Rod)	PARTIAL - COMPLETE INHIBITION	---	---		++	• Opaque/tan-gray • Convex • Glossy • Entire • 1-3mm	
Alternaria spp.	• Catalase (+) ◆ • Ascomycete	+	• Downy to wooly; flat • Grayish, short, aerial hyphae • later becomes greenish black or olive brown with a light border • 3-9cm			++	• Downy to wooly; flat • Grayish, short, aerial hyphae • later becomes greenish black or olive brown with a light border • 3-9cm	
Aspergillus niger	• Catalase (+) ◆ • Ascomycete	+	• Granular • Jet black conidia w/yellow/gray hyphae • 3-9cm			+++	• Granular • White, w jet black fruiting bodies w/ yellow/gray hyphae • 3-9cm	

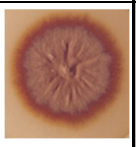
For *in vitro* diagnostic use only. This product should be used only by adequately trained personnel with knowledge of microbiological techniques in the laboratory.

ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Aspergillus flavus	• Catalase (+) ◆ • Ascomycete	+	• Granular to wooly • Yellow, yellow-green or yellow-brown pigment • 3-9cm			+++	• Granular to wooly • Yellow, yellow-green or yellow-brown pigment • 3-9cm	
Aspergillus fumagatus	• Catalase (+) ◆ • Ascomycete	+	• Granular to cottony • Blue-green, green-gray, green-brown pigment • 3-9cm			+++	• Granular to cottony • Blue-green, green-gray, green-brown pigment • 3-9cm	
Aspergillus terreus	• Catalase (+) ◆ • Ascomycete	+	• Granular, radially rugose (wrinkled) • Cinnamon buff, brown pigment • 3-9cm			+++	• Granular, radially rugose (wrinkled) • Cinnamon buff, brown pigment • 3-9cm	
Botrytis spp.	• Catalase (+) ◆ • Ascomycete	+	• Wooly • white, grey/brown pigment • 3-9cm			+++	• Wooly • white, grey/brown pigment • 3-9cm	
Candida albicans	• Catalase (+) ◆ • Ascomycete	+++	• Cream • Convex • Glossy • Entire • 1-2mm			+++	• Pink • Spreading • 6mm	
Chaetomium spp.	• Catalase (+) ◆ • Ascomycete	++	• Wooly • white, grey/olive pigment • 3-5cm			+++	• Wooly • white, grey/olive pigment • 3-5cm	

ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Cladosporium spp.	• Catalase (+) ◆ • Ascomycete	+	• Slow-growing • Mostly olivaceous-brown to blackish but also sometimes grey, buff or brown, suede-like to floccise (wooly), often becoming powdery due to the production of abundant conidia • Vegetative hyphae, coniciophores and conidia are equally pigmented			+	• Slow-growing • Mostly olivaceous-brown to blackish but also sometimes grey, buff or brown, suede-like to floccise (wooly), often becoming powdery due to the production of abundant conidia • Vegetative hyphae, coniciophores and conidia are equally pigmented	
Epicoccum spp.	• Catalase (+) ◆ • Ascomycete	+++	• Wooly/Cottony/ Felty • yellow, orange, red, brown pigment • 3-5+cm			+++	• Wooly/Cottony/ Felty • Purplish / black • 3-5+cm	
Escherichia coli	• Lactose (+) • Indole (+) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod)	+++	• Translucent/ translucent; spreading • Convex, glossy, moist • Entire • 1-2 mm			INHIBITED	---	---
Enterobacter aerogenes	• Lactose (+) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Clear to amber • Convex • Glossy • Entire • 2-4mm			++	• Pink to Red • Convex • Glossy • Entire • 2-4mm	
Fusarium spp.	• Catalase (+) ◆ • Ascomycete	+++	• Fast-growing (4.5 cm in 4 days) • Wooly, white to yellow, pink, red or purple shades • 4+ cm			+++	• Fast-growing (4.5 cm in 4 days) • Wooly, white to yellow, pink, red or purple shades • 4+ cm	

ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Microsporum spp.	• Catalase (+) ◆ • Ascomycete	+	• Glaborous (smooth)/Downy/ Wooly/Powdery • White at first, later becoming grayish yellow to blue green with age • Wrinkled w/ age • 1-9cm			+	• Glaborous (smooth)/Downy/ Wooly/Powdery • White at first, later becoming grayish yellow to blue green with age • Wrinkled w/ age • 1-9cm	
Muccor spp.	• Catalase (+) ◆ • Zygomycete	+	• Wooly, fast-growing • Initially white, the white-yellow to various shades of grey to green showing lollipop sporangia (60-300 µm) • 3-9+ cm			+	• Wooly, fast-growing • Initially white, the white-yellow to various shades of grey to green showing lollipop sporangia (60-300 µm) • 3-9+ cm	
Penicillium chrysogenum (notatum)	• Catalase (+) ◆ • Ascomycete	++	• Granular/wooly, flat • Initially white, then various shades of green, blue-green, or white-green pigment • 3-9+ cm			++	• Granular/wooly, flat • Initially white, then various shades of green, blue-green, or white-green pigment • 3-9+ cm	
Penicillium roqueforti	• Catalase (+) ◆ • Ascomycete	++	• Granular, velvet-like, flat • Initially white, then various shades of green, blue-green pigment • 3-9+ cm			++	• Granular, velvet-like, flat • Initially white, then various shades of green, blue-green pigment • 3-9+ cm	
Penicillium digitatum	• Catalase (+) ◆ • Ascomycete	+++	• Suede-like, wooly • White at first, later becoming green with age • 3-9cm			+++	• Suede-like, wooly • White at first, later becoming green with age • 3-9cm	
Pithomyces spp.	• Catalase (+) ◆ • Ascomycete	++	• Powdery • Pale/dark grey or brown pigment • 3-9cm			+++	• Powdery • Pale/dark grey or brown pigment • 3-9cm	

ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Pseudomomas aeruginosa	• Lactose (-) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod • Fluoresces blue under long-wave UV light (400-nm)	+++	• Transparent to amber • Circular to irregular, spreading • Slightly convex, glossy • Entire • 0.2-0.5 mm			INHIBITED	---	---
Pseudomomas fluorescens	• Lactose (-) • Indole (-) ◆ • Oxidase (+) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod • Fluoresces blue-green under long-wave UV light (400-nm)	+++	• Dull amber to yellow • Irregular • Umbonate, glossy, butryous • Undulate • 4-6 mm			+/-	• Red, pink • Irregular; Spreading to confluent • 2-4mm	
Rhizopus spp.	• Catalase (+) ◆ • Zygomycete	+++	• Cottony • white to blackish grey (black fruiting bodies) • 3-9cm			+++	• Cottony • white to blackish grey (black fruiting bodies) • 3-9cm	
Saccharomyces cerevisiae	• Catalase (+) ◆ • Ascomycete	+++	• Transparent to white to cream • Punctiform • Convex, glossy • Enitre • 0.1-0.5 mm			+++	• White to pink • Punctiform/ circular • Convex, dull • Entire • 0.1-0.5 mm	
Salmonella typhimurium	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+++	• Transparent • Circular • Semi-convex • Entire • 0.5 - 1.0mm			INHIBITED	---	---
Salmonella enteritidis	• Lactose (-) • Indole (-) ◆ • Oxidase (-) ◆ • Catalase (+) ◆ • Urease (-) ◆ • Gram (-) Rod	+	• Transparent • Circular • Semi-convex • Entire • 0.5 - 1.0mm			INHIBITED	---	---

ORGANISM		TSA				RB		
ORGANISM	**PHYSIOLOGY** ◆ Precision Test Strip Available	**GROWTH**	**COLONY**	**IMAGE**		**GROWTH**	**COLONY**	**IMAGE**
Stachybotrys spp.	• Catalase (+) ◆ • Ascomycete	++	• Wooly • black (sometimes: white, pink, orange) • 3-9cm			++	• Wooly • black (sometimes: white, pink, orange) • 3-9cm	
Torula spp.	• Catalase (+) ◆ • Ascomycete	+	• White • Punctiform • Convex, glossy • Entire • 0.1-0.5 mm			+	• White • Punctiform/ circular • Raised • Entire to irregular • 0.1-0.5 mm	
Trichoderma spp.	• Catalase (+) ◆ • Ascomycete	++	• Cottony • White/later scattered green or yellow-green patches (rings) • 3-9cm			++	• Cottony • White/later scattered green or yellow-green patches (rings) • 3-9cm	
Trichophyton spp.	• Catalase (+) ◆ • Ascomycete	+	• Wooly with indented boarders • White to brownish-tan pigment • 3-9cm			+	• Wooly with indented borders • White to brownish-tan pigment • 3-9cm	

+++ = very rich, luxurious growth expected
++ = grows
+ = grows slightly
+/- = may grow; may be inhibited

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:

Catalase Test	Catalase enzyme will react with hydrogen peroxide to produce oxygen if the bacteria is catalase positive.
Lactose Test	Lactose positive bacteria can ferment available lactose in the agar producing an acid which lowers the pH. Lactose negative bacteria are non-fermenting.
Indole Test	Biochemical test to determine the ability of an organism to split indole from the amino acid tryptophan. *P. vulgaris* is indole positive while *P. mirabilis* is indole negative.
Oxidase Test	Oxidase positive bacteria contain cytochrome c oxidase which will turn an indicator dark blue. In contact with oxidase negative bacteria, the indicator will remain colorless.
Urease Test	Bacteria containing urease will hydrolyze urea to ammonia and carbon dioxide causing an alkaline environment which changes the color of a pH indicator from yellow to fuchsia.
β-D-Glucoronidase Reaction	The presence of *E. coli* is determined when both β-D-Glucoronidase and Indole are positive, and the organism is gram negative.
Gram Staining	A method for differentiating bacteria into two groups – gram positive and gram negative – based on the chemical and physical properties of their cell walls. Often the first step in identifying bacteria.