Call: 800-344-4100
Email: tech@lamotte.com

TSA/RB

CODE 5552

USE

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

APPLICATION

TSA is commonly used as a maintenance medium for culture collections, and testing bacterial contaminants in cosmetics. Rose Bengal Agar is recommended in Standard Methods for the enumeration of yeasts and molds from food and water.

PADDLE AGARS

Note: Side 1 of each paddle is marked with an indented laser line.

Side 1: Trypticase Soy Agar (TSA) – (Color: Off-White) Tryptic Soy Agar is an enriched media, suitable to support fastidious heterotrophs and to facilitate vigorous growth of aerobic and anaerobic microorganisms.

Side 2: Rose Bengal Agar (RB) – (Color: Pink) Selective medium for the enumeration of yeasts and molds.

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.

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.

AGAR VERIFICATION

These agars have been verified by EMSL Analytical, Inc. using E. coli and E. faecalis cultures. Documentation available upon request.

SAMPLING

LIQUID SAMPLING PROTOCOL

DIRECT IMMERSION PROTOCOL for Low Viscosity Liquids

1. Twist to remove paddle from vial. Do not touch agar surfaces.
2. Fill vial to 40 mL fill line with the liquid to be sampled and immerse paddle or immerse paddle directly in the sample. Both agar surfaces must be completely contacted. Allow at least 15 second contact time (30 seconds is optimal).
3. Remove paddle. Allow liquid to drain off both agar surfaces.
4. Replace paddle in vial.
5. Incubate.

SPREAD PROTOCOL for High Viscosity Liquids

1. Twist to remove paddle from vial. Do not touch agar surfaces.
2. Hold the contact agar surface on a horizontal plane. Deposit the liquid sample as a single drop approximately 1 cm from the handle boundary.
3. Position a sterile glass rod between the handle and the drop of sample. Bring the rod in contact with the drop to create a meniscus. Drag the rod over the agar surface toward the tip of the paddle.
4. Replace paddle in vial.
5. Incubate.

SURFACE SAMPLING PROTOCOL

Recovery Rate is about 50%

1. Twist paddle to remove from vial. Do not touch agar surfaces.
2. Touch the paddle surface (10 cm^2) to two different areas of the test surface to cover a total of 20 cm^2. Or touch the paddle to the surface once and multiply the colony count by 2.
3. Allow 15 second contact time.
4. Replace the paddle in the vial.
5. Incubate

AIR SAMPLING PROTOCOL

1. Twist to remove paddle from vial. Do not touch agar surfaces.
2. Invert paddle and insert the circular cap.
3. Expose for 15 minutes.
4. Replace paddle in vial.
5. Incubate.

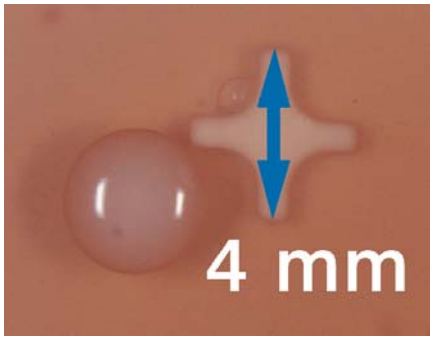
INCUBATION

Incubation of Paddle Growth	Incubation Temperature	Examine at:
Total Coliform / Bacteria	35 ± 2°C	24 to 48 hours
Total Coliform / Bacteria	Room Temperature	Up to 5 days
Yeast / Mold	25 to 30°C	48 hours up to 120 hours (5 days)
Yeast / Mold	Room Temperature	Up to 7 days

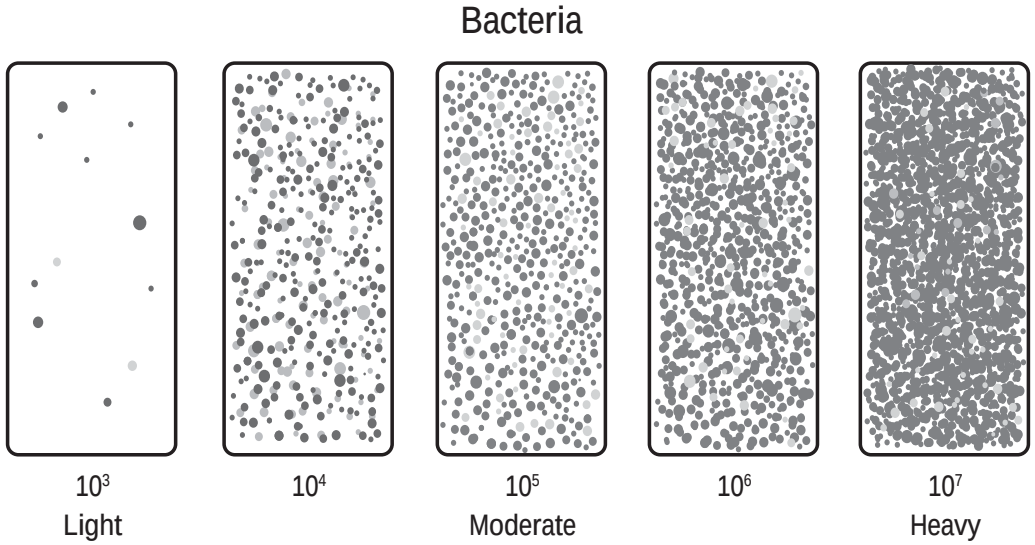
Note: Incubation of bacteria after 48 hours may produce confluent growth making enumeration more difficult.

COLONY MEASURING

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



ENUMERATION



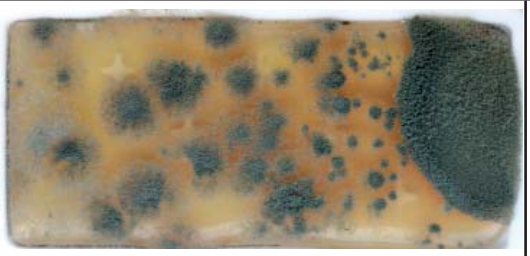
Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold and Mildew growth. Mold and mildew colony growth is more confluent than bacterial growth and therefore more difficult to quantify. Use Light, Moderate, and Heavy for surface and air testing.

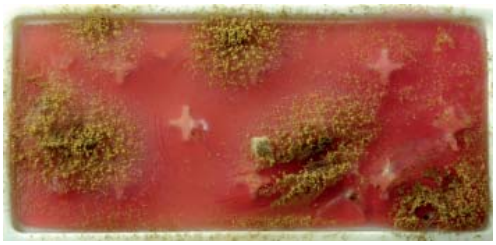
DISPOSAL

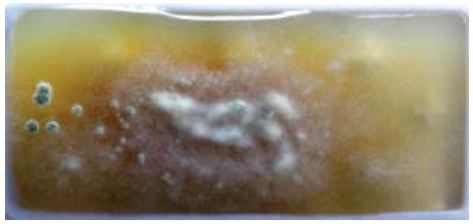
Twist to remove paddle from vial. Fill vial to 40 mL fill line with 1:9 dilution of household bleach (5.25% sodium hypochlorite). Replace paddle in vial. Allow 15 minute contact time. Remove paddle. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen cap and microwave for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

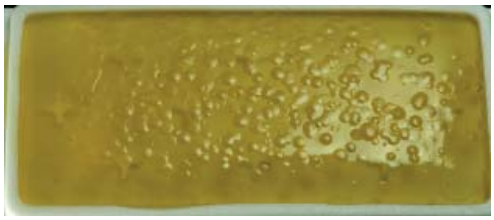
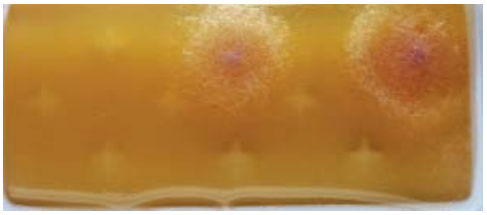
IDENTIFICATION

An organism with Growth +++ will grow very well (non-fastidious) on the indicated media. An organism with Growth + is less likely to grow (fastidious), especially if crowded out by Growth +++ organisms. The media may not contain all of the nutrients that a Growth + organism needs in order to thrive.

Organism	Tryptic Soy (TSA) Agar	Rose Bengal (RB) Agar
Actinomyces bovis	PARTIAL TO COMPLETE INHIBITION	Growth: ++ Colony: Opaque/tan-grey, convex, entire, glossy, 1-3 mm
Alternaria spp.	 Growth: +++ Colony: Suede-like, fast-growing, initially white or yellow-orange, becoming black, grayish-green, olive-green, or grayish, 3-9+ cm (confluent growth)	 Growth: +++ Colony: Suede-like to wooly, 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 - 9+ cm (confluent growth)
Aspergillus niger	 Growth: +++ Colony: Woolly and/or felt-like, forms a carpet, initially white later with jet black fruiting bodies (sporangia), fast growing (4.5 cm in 4 days), 3 - 9+ cm (confluent growth)	 Growth: +++ Colony: Woolly and/or felt-like, forms a carpet, initially white later with jet black fruiting bodies (sporangia), Fast growing (4.5 cm in 4 days) 3 - 9+ cm (confluent growth)

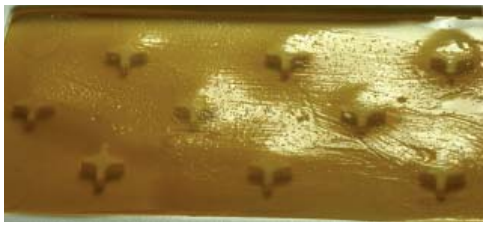
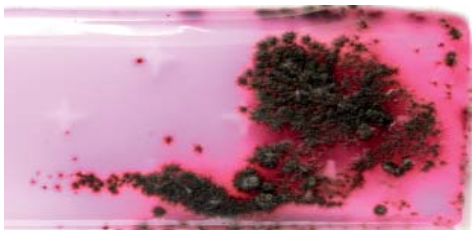
Aspergillus flavus	 Growth: ++ Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9+ cm (confluent growth)	 Growth: +++ Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9+ cm (confluent growth)
Aspergillus fumigatus	 Growth: ++ Colony: Felt-like, initially white to green or blue-green fruiting bodies, forms a carpet, 3-9+ cm (confluent growth)	 Growth: +++ Colony: Suede-like, initially white to green or blue-green fruiting bodies, forms a carpet, 3-9+ cm (confluent growth)
Aspergillus terreus	 Growth: ++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9+ cm (confluent growth)	 Growth: +++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9+ cm (confluent growth)
Bacillus spp.	 Growth: +++ Colony: Translucent/opaque (dull), circular, rough (wrinkled, leathery centers), flat to raised, lobate, 2-5 mm	 Growth: ++ Colony: Translucent to pink, circular to irregular, flat to raised, entire, 2-5 mm

Botrytis spp.	Growth: + Colony: Wooly, white/grey/brown, 3-9 cm	Growth: ++ Colony: Transluscent to pink, circular to irregular, flat to raised, entire, 2-5 mm
Candida albicans	 Growth: +++ Colony: Cream, convex, entire, glossy, 1-2 mm	 Growth: +++ Colony: White to pink, circular, convex, dull, entire, 0.1 - 0.5 mm
Chaetomium spp.	 Growth: ++ Colony: Wooly (mat-like), initially cottony-white turning to olive-green, fruiting bodies (Perithecia) appear as olive-green cockleburs, 3-5+ cm (confluent growth)	 Growth: +++ Colony: Suede-like to wooly, initially white, later globular (roundish) gray or olive areas/ structures (perithecia) looking like cockleburs. 3-5+ cm (confluent growth)
Cladosporium spp.	 Growth: +++ Conlony: Suede-like to wooly, often becoming powdery due to the production of abundant conidia, slow-growing, white turning to olive-brown, buff, or brown, forms a carpet, 3-9+ cm (confluent growth)	 Growth: + Colony: Suede-like to wooly, often becoming powdery due to the production of abundant conidia, slow-growing, white turning to olive-brown, buff, or brown, forms a carpet 3 - 9+ cm (confluent growth)
Epicoccum spp.	Growth: +++ Colony: Wooly, cottony, felty, yellow/orange/ red, 3-5 cm	Growth: +++ Colony: Wooly, cottony, felty, yellow/orange/ red, 3-5 cm

Escherichia coli	 Growth: + ++ Colony: Transparent, spreading, circular, convex, glossy, entire, 1-2 mm	INHIBITED
Enterobacter aerogenes	 Growth: +++ Colony: Transparent, circular to slightly irregular, convex, glossy, butyrous, entire 0.1 - 0.5 mm	INHIBITED
Fusarium spp.	 Growth + ++ Colony: Wooly, initially white with later yellow, pink, red, or purple or pale brown coloring. Fast-growing, 3-9+ cm (confluent growth)	 Growth: + ++ Colony: Wooly, initially white with later yellow, pink, red, or purple or pale brown coloring. Fast-growing, 3-9+ cm (confluent growth)
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, initially white, then white-yellow to various shades of gray to green with lolipop fruiting bodies (sporangia), fast growing, 3 - 9+ cm (confluent growth)	 Growth: ++ Colony: Wooly, initially white, then white-yellow to various shades of gray to green with lolipop fruiting bodies (sporangia), fast growing, 3 - 9+ cm (confluent growth)
Penicillium chrysogenum (notatum)	 Growth: ++ Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-9+ cm (confluent growth)	 Growth: ++ Colony: Granular, velvety/powdery, flat, initially white, then various shades of green-blue, green, or yellow-green, 3-9+ cm (confluent growth)
Penicilium roqueforti	 Growth: ++ Colony: Granular, velvet-like, flat, initially white, then various shades of green, blue-green pigment, 3-9+ cm (confluent growth)	 Growth: + Colony: Granular, velvet-like, flat, initially white, then various shades of green, blue-green pigment, 3- 9+ cm (confluent growth)
Penicillium digittum	Growth: +++ Colony: Suede-like, wooly, initially white, then various shades of olive green, 3-9+ cm (confluent growth)	Growth: +++ Colony: Suede-like, wooly, initially white, then various shades of olive green, 3-9+ cm (confluent growth)

Pithomyces spp.	 Growth: ++ Colony: Powdery, pale/dark gray, brown to olive green pigment, lighter outer ring with center bullseye, 2-9+ cm (confluent growth)	Growth: +++ Colony: Powdery, pale/dark gray, brown to olive green pigment, lighter outer ring with center bullseye, 2-9+ cm (confluent growth)
Pseudomonas aeruginosa	 Growth: +++ Colony: Translucent to amber, circular to irregular, spreading, raised to slightly convex, glossy; entire, 0.5-2+ mm	INHIBITED
Pseudomonas fluorescens	 Growth: +++ Colony: Translucent to amber (with darker center and clear margin), irregular (spreading), convex to umbonate, butyrous, glossy, undulate, 2-4+ mm	 Growth: + Colony: Translucent, pinkish, or amber, irregular, raised, undulate, 2 - 4+ mm
Rhizopus stolonifer	 Growth: +++ Colony: Dense, cottony growth; initially white, turning to gray with black fruiting bodies (sporangia), 3-9+ cm (confluent growth), fast-growing	 Growth: +++ Colony: Dense, cottony growth; initially white, turning to gray with black fruiting bodies (sporangia), 3-9+ cm (confluent growth), fast-growing

Saccharomyces cerevisiae	 Growth: +++ Colony: Translucent to white or cream, covex, entire, glossy, (may be dull), 0.1-1+ mm	 Growth: +++ Colony: Translucent to white or cream, convex, entire, glossy (may be dull), 0.1-0.5 mm (punctiform)
Salmonella typhimurium	 Growth: ++ Colony: Transparent to very light amber, circular to irregular, umbonate, entire 0.5-1.0 mm	INHIBITED
Salmonella (serotype) enteriditis	 Growth: ++ Colony: Transparent to very light amber, circular to irregular, umbonate, entire, 0.5-1.0 mm	INHIBITED
Stachybotrys spp.	 Growth: ++ Colony: Wooly, black (sometimes white, pink, orange) with lighter center, 3-9+ cm	 Growth: ++ Colony: Wooly, black (sometimes white, pink, orange) with lighter center, 3-9+ cm

Torula spp.	 Growth: +++ Colony: White, opaque, viscous, convex, entire, glossy, 0.1 - 0.5 mm (punctiform)	 Growth: +++ Colony: White, opaque, viscous, convex, entire, glossy, 0.1 - 0.5 mm (punctiform)
Trichoderma spp.	 Growth: ++ Colony: Cottony, white, later scattered green or yellow-green patches (rings), 3-9+ cm (confluent growth)	Growth: ++ Colony: Cottony, white, later scattered green or yellow-green patches (rings), 3-9+ cm (confluent growth)
Trichophyton spp.	 Growth: ++ Colony: Wooly with indented boarders. Initially white, with brownish/tan pigmentation, outer darker ring, indentations like spokes on wheel, 3-9+ cm	 Growth: ++ Colony: Wooly, initially white, with brownish/ tan pigmentation, outer darker ring, 3-9+ cm

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.