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TSA/RB MICROSLIDE® TECHNICAL DOCUMENT

Distributed by:

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Index

TSA/RB

CODE: M-TSA/RB

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

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.

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

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 now allow paddles to freeze. 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.

AGAR VERIFICATION

These agars have been verified by EMSL Analytical, Inc. using *P. commune* and *C. albicans* cultures. Documentation available upon request.

SAMPLING

SURFACE Sampling Protocol

1. Remove the paddle from the vial. Do not touch the agar surfaces.
2. To assure an accurate area recovery, contact the paddle to 20^2cm of the surface by contacting the surface twice in separate 10^2cm areas.
3. Replace paddle in vial.
4. Incubate.

LIQUID Sampling Protocol

DIRECT IMMERSION PROTOCOL – low viscous liquids

1. Mix liquid test sample.
2. Remove the paddle from the vial. 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 (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.

SPREAD Protocol – high viscous liquids

1. Mix liquid test sample.
2. Remove paddle from vial. Do not touch the agar surfaces.
3. Holding the contact agar surface on a horizontal plane, deposit volume as a single drop approximately 1cm from the handle boundary (Figure 1).
4. Position a sterile glass rod on the "handle" side of the drop 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.

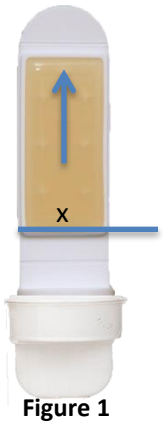


Figure 1

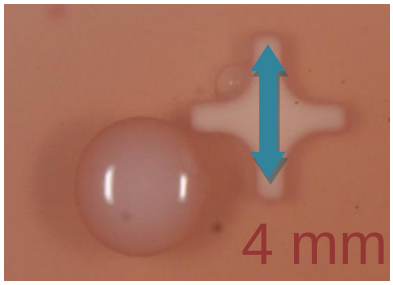
INCUBATION

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

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

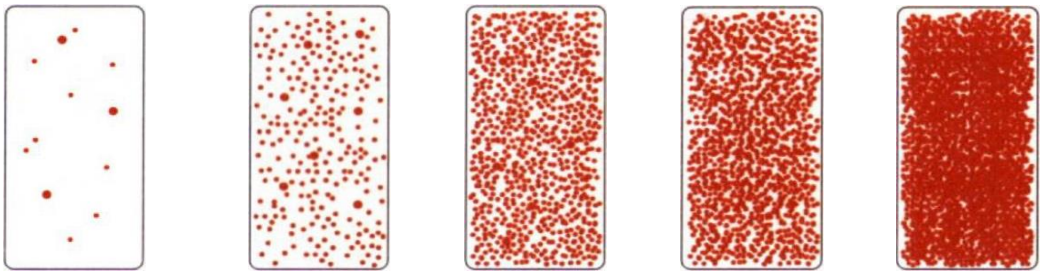
COLONY MEASURING

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

Bacteria CFU/mL



10^3 cfu/mL	**10^4 cfu/mL**	**10^5 cfu/mL**	**10^6 cfu/mL**	**10^7 cfu/mL**
(1,000)	(10,000)	(100,000)	(1,000,000)	(10,000,000)
(Light)		**(Moderate)**		**(Heavy)**

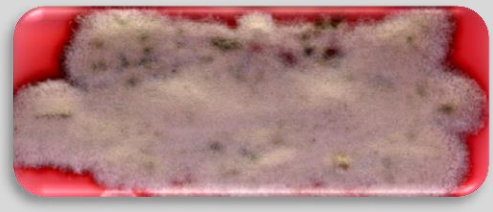
Note: Estimation of lower counts is possible, but statistically difficult to justify. Use Light, Moderate and Heavy for Mold growth and surface testing.

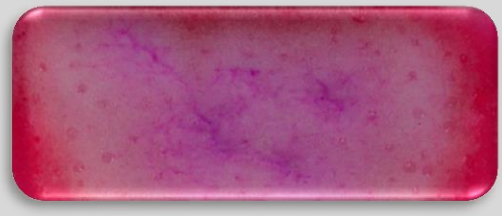
DISPOSAL

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.

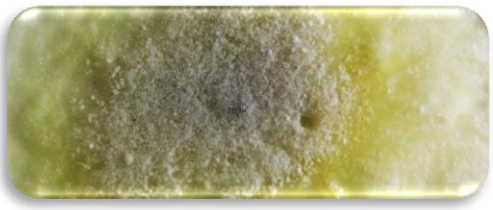
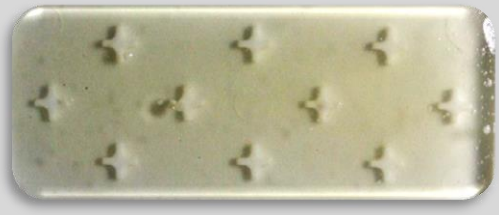
IDENTIFICATION

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: Suede-like, fast-growing, initially white or yellow-orange, becoming black, grayish-green, olive-green, or grayish, 3-9cm+ (confluent growth)	Growth: ++ Colony: Suede-like to woolly, initially white to yellow-orange, becoming black to olive-green or grayish, or grayish-green, umbonate with lighter center area, condication (rings), fast-growing, 3-9cm+ (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 (4.5cm in 4 days), 3-9cm+ (confluent growth)	 Growth: +++ Colony: Woolly and/or felt-like, forms a carpet, initially white later with jet black fruiting bodies (sporangia), fast-growing (4.5cm in 4 days), 3-9cm+ (confluent growth)
Aspergillus flavus	Growth: + Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm+ (confluent growth)	Growth: +++ Colony: Granular to wooly, yellow, yellow-green, or yellow-brown, 3-9cm+ (confluent growth)
Aspergillus fumigatus	Growth: ++ Colony: Felt-like, forms a carpet, initially white to green or blue-green fruiting bodies, 3-9cm+ (confluent growth)	Growth: +++ Colony: Felt-like, forms a carpet, initialy white to green or blue-green fruiting bodies, 3-9cm+ (confluent growth)
Aspergillus terreus	Growth: ++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm+ (confluent growth)	Growth: +++ Colony: Granular, radially rugose (wrinkled), cinnamon buff/brown, 3-9cm+ (confluent growth)
Bacillus spp.	 Growth: +++ Colony: Translucent/Opaque (dull), circular, rough (wrinkled, leathery centers), flat to raised, lobate, 2-5mm	 Growth: ++ Colony: Translucent to pink, circular to irregular, flat to raised, entire, 2-5mm

Botrytis spp.	Growth: + Colony: Wooly, white/grey/brown, 3-9cm	Growth: +++ Colony: Wooly, white/grey/brown pigment, 3-9cm
Candida albicans	 Growth: +++ Colony: Cream, CVEG, 1-2mm	 Growth: +++ Colony: White to pink, circular, convex, dull, entire, 0.1-0.5mm
Chaetomium spp.	Growth: ++ Colony: Wooly (mat-like), initially cottony-white turning olive-green, fruiting bodies (perithecia) appear as olive-green cockleburs, 3-5cm+ (confluent growth)	Growth: +++ Colony: Suede-like to Woolly, initially white, later globular (roundish) gray or olive areas / structures (perithecia) looking like cockleburs, 3-5cm+ (confluent growth)
Cladosporium spp.	 Growth: + Colony: Suede-like to woolly, often becoming powdery due to the production of abundant conidia, forms a carpet, white turning olive-brown, buff, or brown, slow-growing, 3-9cm+ (confluent growth)	 Growth: + Colony: Suede-like to woolly, often becoming powdery due to the production of abundant conidia, forms a carpet, white turning olive-brown, buff, or brown, slow-growing, 3-9cm+ (confluent growth)
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: Transparent, spreading, Convex, glossy, entire, 1-2mm	INHIBITED

Enterobacter aerogenes	 Growth: +++ Colony: Transparent, circular to slightly irregular, convex, glossy, butyrous, entire, 0.1-0.5mm	INHIBITED
Fusarium spp.	 Growth: +++ Colony: Wooly, initially white, later with yellow, pink, red, purple or pale brown coloring, fast-growing, 3-9cm+ (confluent growth)	 Growth: +++ Colony: Wooly, initially white, later with yellow, pink, red, purple or pale brown coloring, fast-growing, 3-9cm+ (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: Woolly, initially white, then white-yellow to various shades of gray to green with lollipop fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth)	 Growth: + Colony: Woolly, initially white, then white-yellow to various shades of gray to green with lollipop fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth)
Penicillium chrysogenum (notatum)	 Growth: ++ Colony: Granular, velvety/powdery, flat,	 Growth: ++ Colony: Granular, velvety/powdery, flat,

	initially white, then various shades of green-blue, green, or yellow-green, 3-9cm+ (confluent growth)	initially white, then various shades of green-blue, green, or yellow-green, 3-9cm+ (confluent growth)
Penicilium roqueforti	 Growth: ++ Colony: Granular, velvet-like, flat, initially white then various shades of green, blue-green pigment, 3-9cm+ (confluent growth)	 Growth: ++ Colony: Granular, velvet-like, flat, initially white then various shades of green, blue-green pigment, 3-9cm+ (confluent growth)
Penicillium digittum	Growth: +++ Colony: Suede-like, woolly, initially white, then various shades of olive green, 3-9cm+ (confluent growth)	Growth: +++ Colony: Suede-like, woolly, initially white, then various shades of olive green, 3-9cm+ (confluent growth)
Pithomyces spp.	Growth: ++ Colony: Powdery, pale/dark grey or brown to olive green pigment, lighter outer ring with center bullseye, 2-9cm+ (confluent growth)	Growth: +++ Colony: Powdery, pale/dark grey or brown to olive green pigment, lighter outer ring with center bullseye, 2-9cm+ (confluent growth)
Pseudomonas aeruginosa	 Growth: +++ Colony: Translucent to amber, circular to irregular, spreading, raised to slightly convex, glossy, entire, 0.5-2.0mm+	INHIBITED
Pseudomonas fluorescens	 Growth: +++ Colony: Translucent to amber (with darker center and clear margin), irregular (spreading), convex to umbonate, butyrous, glossy, undulate, 2-4mm+	 Growth: + Colony: Translucent, pinkish, or amber, irregular, raised, undulate, 2-4mm+

Rhizous spp.	 Growth: +++ Colony: Dense, cottony growth, initially white, turning to gray with black fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth)	 Growth: +++ Colony: Dense, cottony growth, initially white, turning to gray with black fruiting bodies (sporangia), fast-growing, 3-9cm+ (confluent growth)
Saccharomyces cerevisiae	 Growth: +++ Colony: Translucent to white or cream, CVEG (may be dull), 0.1-1.0mm+	 Growth: +++ Colony: Translucent to white or cream, CVEG (may be dull), 0.1-0.5mm (punctiform)
Salmonella typhimurium	Growth: ++ Colony: Transparent to very light amber, circular to irregular, umbonate, entire, 0.5-1.0mm	INHIBITED
Salmonella enteriditis	 Growth: ++ Colony: Transparent to very light amber, circular to irregular, umbonate, entire, 0.5-1.0mm	INHIBITED
Stachybotrys spp.	Growth: ++ Colony: Woolly, black (sometimes white, pink, orange) with lighter center, 3-9cm+	Growth: ++ Colony: Woolly, black (sometimes white, pink, orange) with lighter center, 3-9cm+
Torula spp.	 Growth: +++	 Growth: +++

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

GLOSSARY

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