NUT/MAC

CODE 5553

USE

Isolation and differentiation of Gram (-) enteric bacilli. Coliform Testing/Recovering of Stressed Coliforms

APPLICATION

In total coliform testing, the coliform organisms tested for include: total coliform, fecal coliform, and E. coli (Escherichia coli). Detection of fecal coliforms (a subset of total coliforms) or Escherichia coli (a subset of fecal coliforms) can indicate the potential presence of waterborne pathogens associated with fecal contamination.[1]

PADDLE AGARS

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

Side 1: Nutrient-TTC Agar (NUT) – (Color: Yellow) General purpose (relatively non-selective) medium, which will support the growth of a wide variety of organisms. Suitable for cultivation of both aerobes and anaerobes. Aerobic coliform bacteria can be detected by their ability to reduce the Triphenyl tetrazolium chloride (TTC) dye to a red-colored formazan dye. Bacterial colonies appear as red dots on an otherwise yellow medium.

Note: Paddle color is normally LIGHT YELLOW when the NUT agar is cast (about pH 6.0). Some microorganism growth (even before colonies are OBSERVABLE) will shift the pH from an acidic level to a more alkaline level (pH 7.0 or higher) – turning the agar a light green.

Side 2: MacConkey Agar (MAC) – (Color: Brownish-pink) Both selective AND differential; used to differentiate between Gram negative bacteria while inhibiting the growth of most Gram positive bacteria. The medium also differentiates between lactose-fermenting coliforms (Lac (+)) and lactose non-fermenters (Lac (-)), which include potential pathogens.

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.

[1]United State Pharmacopeial Convention. 2007 The United States Pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacoeial Convention, Rockville, MD.

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.

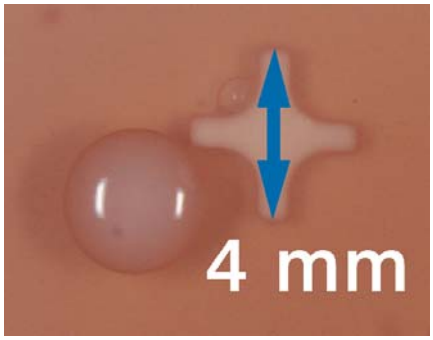
INCUBATION

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

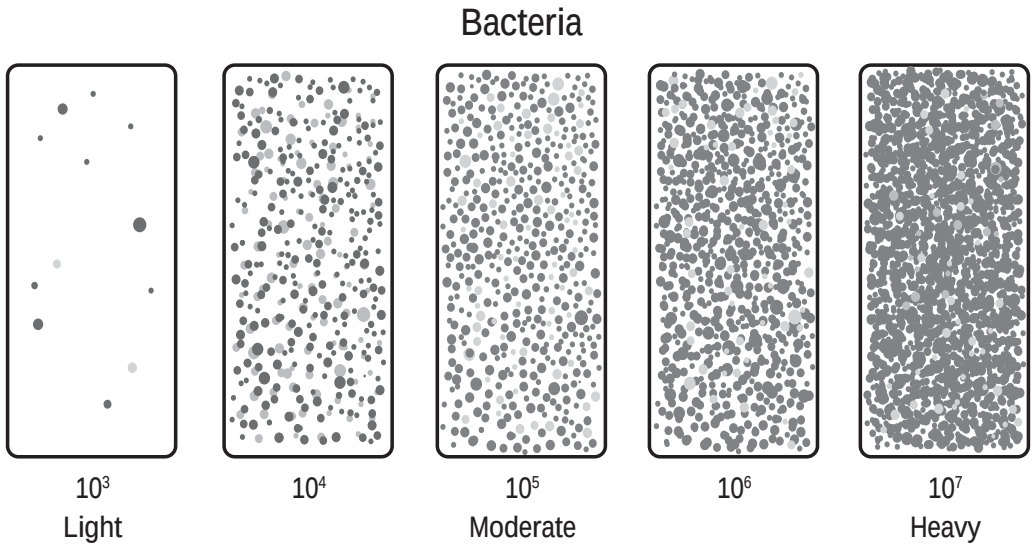
Note: Incubation of bacteria after 48 hours may produce confluent growth making enumeration more difficult.

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.

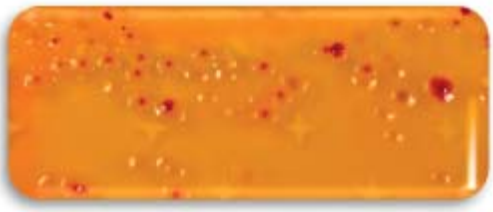
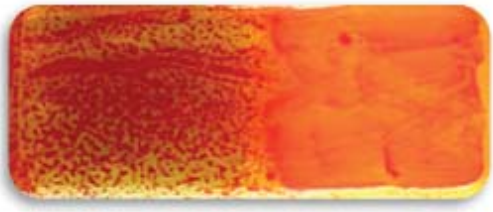
DISPOSAL

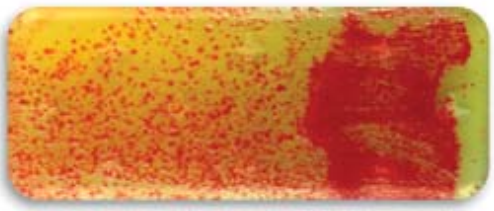
Twist to remove paddle from vial. Fill vial to 40 mL fill line with 1:9 dilution of household bleach (5.25% sodium hypochlorite). Replace paddle in vial. Allow 15 minute contact time. Remove paddle. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen cap and microwave for 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	Nutrient-TTC (NUT) Agar	MacConkey (MAC) Agar
Aspergillus niger	 Growth: +++ Colony: Granular, jet black conidia with yellow/gray hyphae, 3-5++ cm	INHIBITED
Bacillus spp.	 Growth: +++ Colony: Irregular, raised, lobate (wrinkled), opaque, with darker center (bullseye) 2-4+ mm	INHIBITED
Candida albicans	 Growth: +++ Colony: Cream, convex, entire, glossy, 1-2 mm	INHIBITED

Escherichia coli	 Growth: +++ Colony: Yellow/orange/red, convex, entire, glossy, 2-4 mm	 Growth: +++ Colony: Pink/red, convex, entire, glossy, 0.2-0.5 mm
Enterobacter aerogenes	 Growth: +++ Colony: Maroon with transparent margin, convex, entire, glossy, 0.1 - 0.5 mm	 Growth: +++ Colony: Pink, thick, round, raised to low-convex, spreading, 0.1-0.5 mm
Enterococcus spp.	INHIBITED	PARTIAL TO COMPLETE INHIBITION
Klebsiella spp.	 Growth: +++ Colony: Amber/red, spreading, 0.5-1.0 mm	 Growth: +++ Colony: Colorless/light pink, spreading, 0.5-1.0 mm
Proteus spp.	 Growth: +++ Colony: Maroon/red with dark red center and transparent margin; irregular, glistening (swarming - transparent field), raised, undualte, 1-4 mm	 Growth: + Colony: Colorless to yellow, pink/red, circular, wrinkled (flower-like), umbonate, erose, 2-3 mm

Pseudomonas aeruginosa	 Growth: +++ Colony: Maroon with transparent margin, circular to irregular, raised, entire, 1-2 mm	 Growth: +++ Colony: Transparent, convex, entire, glossy 0.1-0.2 mm (punctiform)
Pseudomonas fluorescens	 Growth: +++ Colony: Clear/colorless with grey/dark center, translucent edges, irregular/ spreading to confluent, 2-4 mm	 Growth: +++ Colony: Clear/pink with dark pink center, transluecent edges, irregular edges, 2-4 mm
Salmonella (serotype) enteriditis	 Growth: +++ Colony: Red, full, entire, dull, 0.5-1.0 mm	 Growth: +++ Colony: Gray to white (pearl), circular, umbonate, entire, 1-2 mm
Serratia spp.	 Growth: ++ Colony: Red, full, entire, dull, 0.5-1.0 mm	 Growth: + Colony: Pink, convex, dull, entire, 0.1-0.5 mm (punctiform)

Shigella spp.	 Growth: + Colony: Maroon/red, convex, entire, glossy, 0.5-1.0 mm	 Growth: +++ Colony: Transparent to gray (pearl), circular, raised, dull, entire, 1-2 mm
Staphylococcus aureus	 Growth: + Colony: Red, full, entire, dull, 0.5-1.0 mm	PARTIAL TO INHIBITED GROWTH
Streptococcus spp.	 Growth + + Colony: Maroon (red), convex, entire, glossy, 0.1-0. 5mm	 Growth: + Transparent, circular, umbonate, glistening, entire, 1-2 mm
Streptomyces griseus	 Growth: + Colony: Yellow, full, entire, dull, 0.5-1.0 mm	PARTIAL TO COMPLETE INHIBITION
Gram (+) Bacteria	PARTIAL TO COMPLETE INHIBITION	PARTIAL TO COMPLETE INHIBITION

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.