

NUT-TTC/MAC

Code 5553

Nutrient-TTC Agar (**NUT-TTC**) MacConkey Agar (**MAC**)

USE:

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

Side 1: Nutrient-TTC Agar (NUT-TTC) (yellow) (Side 1 is marked with an indented laser line)

Side 2: MacConkey Agar (MAC) (pink)



APPLICATION

In total coliform testing (TCC), 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 contamination¹.

PADDLE AGARS

Nutrient-TTC Agar (NUT-TTC) –General purpose relatively non-selective agar medium, containing two peptones, which will support the growth of a wide variety of organisms. Suitable for cultivation of both of aerobes and anaerobes. This medium contains the dye, 2,3,5- triphenyltetrazolium chloride (TTC)². Aerobic coliform bacteria species grow on this medium and can be detected by their ability to reduce TTC to a red colored formazan dye. Bacterial colonies appear as red dots on an otherwise clear yellowish medium. Agar is the solidifying agent.

MacConkey Agar (MAC) – 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. Addition to the nutrient agar base of bile salts and crystal violet will inhibit the growth of most Gram-positive bacteria, making MacConkey agar selective. Lactose, a fermentable carbohydrate, and neutral red, a pH indicator, are added to differentiate the lactose positive coliforms from the potentially pathogenic lactose non-fermenters. When lactose is fermented, acid products lower the pH below 6.8, with the resulting colonial growth turning pinkish-red. If an organism is unable to ferment lactose, the colonies will be colorless. Bile salts mixture and crystal violet are the selective agents, inhibiting Gram positive cocci and allowing Gram-negative

¹ United States Pharmacopeial Convention. 2007. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.

² Chapman, G. H. 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. 53:504.



organisms to grow. Sodium chloride maintains the osmotic environment. Agar and a proprietary polymer are the solidifying agents.

CULTURE CONTROLS

10-300 inoculum (CFU)

	Nutrient-TTC Agar	MacConkey Agar
Enterococcus faecalis	GROWTH	INHIBITED
Escherichia coli	GROWTH	GROWTH
Proteus mirabilis	INHIBITED	GROWTH &
		PARTIAL INHIBITION
		OF SWARMING
Salmonella typhimurium	GROWTH	GROWTH

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 cm²) to the test surface twice to cover a 20 cm² area (2 X 10 cm²). Allow 15 second contact time. Replace paddle in vial.

INCUBATION Incubate at 35 ±2C for 18-24 hours.



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		NUT-TTC			MAC			
ORGANISM	PHYSIOLOGY Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE	
Aspergillus niger	• Catalase (+) • Ascomycete	+++	• Granular • Jet black conidia w/ yellow/gray hyphae • 3-5++cm		INHIBITED			
Bacillus spp.	 Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease () ◆ Gram (+) Rod 	+++	Translucent with darker center Irregular, ough Raised Lobate 1-4mm		INHIBITED			
Candida albicans	Catalase (+) Ascomycete	+++	• Cream • Convex • Glossy • Entire • 1-2mm		INHIBITED			
Escherichia coli	 Lactose (+) Indole (+) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod) 	+++	 Transparent/ orange to red center (target); large Circular Umbonate, glossy Entire 2-4 mm 		+++	 Pink/Red Punctiform Convex, glossy, moist Entire 0.20.5 mm 	* + * +	

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ORGANISM		NUT-TTC			MAC			
ORGANISM	PHYSIOLOGY Precision Test Strip Available	GROWTH	COLONY	IMAGE	GROWTH	COLONY	IMAGE	
Enterobacter aerogenes	 Lactose (+) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod 	+++	•Transparent • Convex • Glossy • Entire • 2-4mm	A AMERICA	+++	 Pink Thick, round, raised to low- convex; Spreading 2-3mm 	y and	
Enterococcus spp.	 Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (-) ◆ Urease (-) ◆ Gram (+) Sphere 	INHIBITED			INHIBITED			
Klebsiella spp.	 Lactose (+) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (+) ◆ Gram (-) Rod 	+++	Orange to red with small dark center; transparent margin Circular to irregular Raised to convex; mucoid Entire to undulate 0.5-1.0 mm		+++	White to ivory Circular to irregular Raised to convex, glossy Entire to undulate 0.5-1.0 mm	·+ +	
Proteus spp.	Lactose (-) SEE: INDOLE Oxidase (-) Catalase (+) Urease (+) Gram (-) Rod	INHIBITED	Maroon/red with a dark red center and transparent margin Irregularly; glistening (swarming- transparent field) Raised Undulate/ curled 1-4 mm		+++	Colorless; to yellow to pink/red Circular; wrinkled, flower-like Umbonate Erose 2-4 mm INHIBITED		



ORGANISM		NUT-TTC				MAC			
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)	+++	Maroon to transparent margin Circular Raised, glossy Entire 1-2 mm			+++	Transparent Circular Convex, glossy Undulate 0.5-1.0 mm		
Pseudomomas fluorescens	Lactose (-) Indole (-) Oxidase (+) Catalase (+) Catalase (+) Urease (-) Gram (-) Rod Fluoresces blue- green under long-wave UV light (400-nm)	+++	 Transparent with dull tan center Irregular Raised, glossy, butryous, spreading Entire 2-4 mm 			+++	Transparent Circular Convex Glossy Undulate 0.5-1 mm		
Salmonella typhimurium	 Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (-) Rod 	+++	Maroon, reddish orange Punctiform to circular Semi-convex Entire 0.5 - 1.0mm			+++	Clear, transparent or amber Circular Convex Entire to slightly lobate 1-2mm		
Salmonella enteritidis	Lactose (-) Indole (-) Oxidase (-) Catalase (+) Urease (-) Gram (-) Rod	+	Maroon, reddish orange Punctiform to circular Semi-convex Entire 0.5 - 1.0mm			PARTIAL - Complete Inhibition			
Serratia spp.	 Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (+) ◆ Gram (-) Rod 	+	Orange to maroon with transparent margin Circular Convex, glossy Entire to undulate 0.5-1.0 mm			++	 Pink Punctiform Convex, dull Entire 0.1-0.5 mm 		

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ORGANISM		NUT-TTC				MAC		
ORGANISM	PHYSIOLOGY Precision Test Strip Available	GROWTH	COLONY	IMAGE		GROWTH	COLONY	IMAGE
Shigella spp.	Lactose (-) Indole - mixed Oxidase (-) Catalase (+) Urease (-) Gram (-) Rod	PARTIAL INHIBITION	Maroon.Red Punctiform Convex, glossy Entire 0.5-1.0 mm			+++	 Transparent to gray (pearl) Circular Raised, dull Entire 1-2 mm 	
Staphylococcus aureus	 Lactose (-) Indole (-) ◆ Oxidase (-) ◆ Catalase (+) ◆ Urease (-) ◆ Gram (+) Sphere 	PARTIAL - COMPLETE INHIBITION				PARTIAL - COMPLETE INHIBITION		
Streptococcus spp.	Lactose (+) ◆ Indole (+) ◆ Oxidase (-) ◆ Catalase (-) ◆ Urease (+) ◆ Gram (+) Sphere	PARTIAL - COMPLETE INHIBITION				PARTIAL - COMPLETE INHIBITION		
Gram (+) Bacteria		PARTIAL - Complete Inhibition				PARTIAL - COMPLETE INHIBITION		
+++ = very rich, luxurious growth expected ++ = grows + = grows slightly +/- = may grow; may be inhibited				NOTE: Color may	<i>i</i> change from pir	nk to green.		

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

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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. <i>P. vulgaris</i> is indole positive while <i>P. mirabilis</i> 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 <i>E. coli</i> 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.