PRESUMPTO QUADRANT PLATE

PRODUCT:

Plate Media:

Presumpto Quadrant Plate P3650

PURPOSE:

The Presumpto Quadrant Plate is used for the differentiation and presumptive identification of anaerobic bacteria.

PRINCIPLE:

The Presumpto Quadrant Plate is prepared by the formula of Lombard and Dowell[1] and contains three modifications of Lombard-Dowell (LD) Agar. The first quadrant contains no additives and is used for the establishment of growth and the determination of indole production. The second quadrant contains LD Esculin Agar, and is used to determine esculin hydrolysis, H_2S, and catalase production. The third quadrant contains LD Egg Yolk Agar, and is used to determine proteolysis, lipase, and lecithinase production. The fourth quadrant contains LD Bile Agar and is used to determine the organism's ability to grow in the presence of 2% oxgall and form a precipitate. Other tests which may be performed for the presumptive identification of anaerobes include cellular and colonial morphology, Gram stain reaction, hemolysis, pigment production, and the susceptibility to penicillin, rifampin, and kanamycin. Determination of metabolic by-products may be determined by growth in peptone yeast extract glucose (PYG) broth cultures.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) LD Presumpto Plate, Base Formula, Quadrant I:

Pancreatic Digest of Casein	5.0 g
Yeast Extract	5.0
L-Tryptophan	0.2
Sodium Chloride	2.5
L-Cysteine	0.4
Sodium Sulfite Anhydrous	0.1
Agar	20.0
Vitamin K	1.0 mg
Hemin[1]	10.0 mg

(2) Quadrant II - LD Esculin Agar:
Same as (1) with 1.0 g of Esculin and 0.5 g of Ferric Citrate.

(3) Quadrant III - LD Egg Yolk Agar:
Same as (1) with 2.0 g of Glucose, 5.0 g of Disodium Phosphate, 0.2 ml of 5% Magnesium Sulfate, and 100.0 ml of Egg Yolk Suspension.

(4) Quadrant IV - LD Bile Agar:
Same as (1) with 20.0 g of Oxgall and 1.0 g of Glucose

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: This medium is designed for the presumptive identification of anaerobic organisms. Definitive identification depends on further physiological, biochemical, and/or serological procedures.

The catalase reaction is an aerobic process. It is essential that the plates be exposed to ambient air for a minimum of 30 minutes before this test is performed.

PROCEDURE:*

Specimen Collection: Not applicable since this medium is not for primary isolation. This medium is used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating this medium. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. A turbid suspension (McFarland #1) should be made of the organism to be tested by 1) suspending well-isolated colonies from a pure culture into a suitable broth, or 2) inoculating a single colony to an enriched thioglycollate medium, which is then incubated until the proper turbidity is reached.

Using a small bore disposable pipette, place two drops of the cell suspension or broth culture onto each quadrant or make a quarter-sized inoculum in each quadrant using a swab. Place a sterile, blank, 1/4" diameter paper disk on the LD agar (quadrant I) near the outer periphery of the inoculum. This disk is used for indole detection after incubation of the plates. Inoculate a purity plate and streak for isolation. Incubate anaerobically at 35°C for 48 hours.

Interpretation:

1. **LD Agar:**
   (A) Growth: Note and record the degree of growth on LD Agar (light, moderate, heavy).

   (B) Indole Production: Test for indole production by adding two drops of paradimethylaminocinnamaldehyde(Indole Spot - PML item #R6510) reagent to the paper disk on the medium. The production of a blue or bluish-green color on the disk within 30 seconds is a positive reaction. Development of another color (pink, red, or violet), or no color is considered a negative result.

2. **LD Esculin Agar:**
   (A) Esculin Hydrolysis: A positive test for esculin hydrolysis is indicated by the development of reddish-brown to a dark brown color in the esculin agar surrounding bacterial colonies after exposure to air for at least 5 minutes. A negative reaction is the absence of a dark brown color.

   (B) H_2S Production: Blackening of the bacterial colonies on the esculin agar is an indication of H_2S production. The blackening dissipates very rapidly when exposed to air; therefore, the bacterial growth should be observed for blackening under anaerobic conditions (anaerobic glove box), or immediately after opening the anaerobic jar before prolonged exposure to air. For this reason, this test should be read before the esculin hydrolysis.

   (C) Catalase: To test for hydrogen peroxidase degradation as an indication of catalase production, expose the plates to air for at least 30 minutes, then flood the esculin quadrant with a few drops of 3% hydrogen peroxide. Sustained bubbling after the addition of hydrogen peroxide is interpreted as a positive reaction for catalase. In some cases, rapid bubbling may not be evident until after 30 seconds to a minute. No bubbling of the colonies after the addition of the reagent is indicative of a negative result.

3. **LD Egg Yolk Agar:**
   (A) Lecithinase Production: The formation of a zone of insoluble precipitate in the medium surrounding the bacterial colonies is considered positive for lecithinase production. This is best seen with transmitted light.

   (B) Lipase Production: Examine the plate for the presence of an iridescent sheen or “pearly layer” on the surface of the colonies and on the medium surrounding the bacterial growth (positive). This phenomenon is best demonstrated with reflected light. Plate should be held for up to a week for this reaction.

   (C) Proteolysis Production: Clearing of the medium surrounding the bacterial growth indicates proteolysis.

4. **LD Bile Agar:**
   (A) Compare the degree of bacterial growth on the LD Bile Agar with that of the LD Agar and record growth as less than, equal to, or greater than, the LD agar control.

   (B) Using transmitted light, look for the presence or absence of an insoluble white precipitate underneath and or immediately surrounding the bacterial growth. If in doubt, inspect under a stereo microscope using transmitted light. Increased growth on the agar plate and/or presence of precipitate is indicative of the ability of the organism to grow in the presence of bile.

Refer to the references cited for identification charts and tables.[3,4,5]

Materials Required but Not Provided: Standard microbiological supplies and equipment as well anaerobic growth equipment.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):

	I	II	III	Expected Results: IV
Bacteroides fragilis (25285)	Growth	Esculin (+)	Lecithinase (-)	Growth (>) control
	Indole (-)	H_2S (-)	Lipase (-)	Bile ppt (+)
		Catalase (+)	Proteolysis (-)	
Clostridium perfringens (13124)	Growth	Esculin (-)	Lecithinase (+)	Growth (<) control
	Indole (-)	H_2S (-)	Lipase (-)	Bile ppt (-)
		Catalase (-)	Proteolysis (-)	
Fusobacterium nucleatum (25586)	Growth	Esculin (-)	Lecithinase (-)	Growth (<) control
	Indole (+)	H_2S (-)	Lipase (-)	Bile ppt (-)
		Catalase (-)	Proteolysis (-)	
Clostridium sporogenes (11437)	Growth	Esculin (+)	Lecithinase (-)	Growth (<) control
	Indole (-)	H_2S (-)	Lipase (+)	Bile ppt (-)
		Catalase (-)	Proteolysis (-)	

Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Dowell, V. R., Jr., and G. L. Lombard, *Presumptive Identification of Anaerobic Nonsporeforming Gram Negative Bacilli*, CDC, Atlanta, 1978.
2. Holdeman, L. V., and W. E. C. Moore, *Anaerobic Laboratory Manual*, 4th ed., Virginia Polytechnical Institute, Blacksburg, Va., 1977.
3. Koneman, E.W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 5th ed., J. B. Lippincott, Philadelphia, 1997.
4. Murray, P. R., et al., *Manual of Clinical Microbiology*, 8th ed., American Society for Microbiology, Washington D.C., 2003.
5. Sutter, V. L., et al., *Anaerobic Bacteriology Manual*, 3rd ed., UCLA Dept. of Continuing Education Health Sciences, Los Angeles, 1980.

*For more detailed information, consult appropriate references.

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