

ANAEROBIC CDC #3 QUAD

INTENDED USE

Remel Anaerobic CDC #3 Quad is comprised of solid media recommended for use in qualitative procedures for the presumptive identification of anaerobic bacteria.

SUMMARY AND EXPLANATION

In 1975, the Anaerobic Laboratory at the Center for Disease Control (CDC) developed three quadrant plates derived from Lombard-Dowell (L-D) Agar for use in the identification of anaerobic bacteria.¹ The quadrant plates have been designated by the CDC as Presumptive Plates 1, 2 and 3.² Anaerobic CDC #3 Quad contains L-D Gelatin Agar and three carbohydrate media (L-D Mannitol Agar, L-D Lactose Agar, and L-D Rhamnose Agar). The development and use of the Gelatin Agar was described by Wanderlinder et al.³

PRINCIPLE

Lombard-Dowell Agar Base supplemented with vitamin K and hemin, supports the growth of common anaerobic bacteria. Cystine and sodium sulfite are reducing agents. Quadrant I contains gelatin to test for gelatin hydrolysis. Mercuric chloride (Gelatin Reagent) is added after incubation to precipitate the undigested gelatin in the medium. Quadrants II, III, and IV contain mannitol, lactose, and rhamnose, respectively. The fermentation of these carbohydrates is detected by acid production, which results in the bromthymol blue indicator changing from blue to yellow.

REAGENTS (CLASSICAL FORMULAE)*

Base Medium:

Casein Peptone.....	5.0 g	L-Tryptophan	0.2 g
Yeast Extract.....	5.0 g	Sodium Sulfite	0.1 g
Sodium Chloride.....	2.5 g	Vitamin K	10.0mg
Dextrose.....	1.0 g	Hemin	5.0mg
L-Cystine	0.4 g	Agar.....	20.0 g
		Deminerlized Water	1000.0 ml

pH 7.5 ± 0.2 @ 25°C

The following ingredients are added per liter of medium:

Quadrant I

Gelatin.....4.0 g

Quadrant II

Mannitol.....6.0 g
Bromthymol Blue (1%)2.0ml

Quadrant III

Lactose6.0 g
Bromthymol Blue (1%).....2.0ml

Quadrant IV

Rhamnose6.0 g
Bromthymol Blue (1%).....2.0ml

*Adjusted as required to meet performance standards.

PROCEDURE

1. Implement appropriate procedures to verify that the test isolate is an anaerobe.
2. Prepare an inoculum from a pure culture of the test isolate using Thioglycollate Broth (REF R064732) or suitable alternative equal in density to a #1 McFarland Standard or equivalent (REF R20411).
3. Using a sterile Pasteur pipette, remove a portion of the bacterial suspension and add 1-2 drops to each quadrant. Streak using a sterile loop.
4. Incubate the quad plate anaerobically for 48-72 hours at 33-37°C.
5. After incubation, flood Quadrant I with Gelatin Reagent (REF R21226) and observe for clearing of the agar.
6. Observe Quadrants II, III, and IV for carbohydrate fermentation demonstrated by yellow colonies with yellow zones.

INTERPRETATION OF THE TEST

Quadrant I (Gelatin):

Positive Test - Clearing around colonies after addition of Gelatin Reagent; background remains opaque

Negative Test - Agar remains opaque

Quadrants II, III, and IV (Carbohydrate Fermentation):

Positive Test - Yellow colonies with yellow zone

Negative Test - Growth with no color change

QUALITY CONTROL

All lot numbers of Anaerobic CDC #3 Quad have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS			
		Gelatin	Mannitol	Lactose	Rhamnose
<i>Bacteroides ovatus</i> ATCC® 8483	Anaerobic, 48-72 h @ 33-37°C	-	+	+	+
<i>Clostridium difficile</i> ATCC® 9689	Anaerobic, 48-72 h @ 33-37°C	V	+	-	-
<i>Clostridium sporogenes</i> ATCC® 3584	Anaerobic, 48-72 h @ 33-37°C	+	-	-	-
<i>Fusobacterium mortiferum</i> ATCC® 25557	Anaerobic, 48-72 h @ 33-37°C	-	-	+	-

(Continued on back)

BIBLIOGRAPHY

1. Whaley, D.N., L.S. Wiggs, P.H. Miller, P.U. Srivastava, and J.M. Miller. 1995. J. Clin. Microbiol. 33:1196-1202.
2. Dowell, V.R., Jr. and G.L. Lombard. 1977. Presumptive Identification of Anaerobic Nonsporeforming Gram-Negative Bacilli. U.S. Dept of H.H.S., CDC, Atlanta, GA.
3. Whaley, D.N., V.R. Dowell, Jr., L.M. Wanderlinder, and G.L. Lombard. 1982. J. Clin. Microbiol. 16:224-229.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

ATCC® is a registered trademark of American Type Culture Collection.

IFU 2410, Revised August 21, 2007

Printed in U.S.A.

remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA

General Information: (800) 255-6730 Technical Service: (800) 447-3641 Order Entry: (800) 447-3635

Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128

Website: www.remel.com Email: remel@remel.com