ANAEROBIC GRAM-NEGATIVE ID QUAD

INTENDED USE

Remel Anaerobic Gram-Negative ID Quad is comprised of solid media recommended for use in qualitative procedures for the presumptive identification of anaerobic, gram-negative, nonsporeforming 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 Presumpto Plates 1, 2 and 3.[2] Anaerobic Gram-Negative ID Quad contains L-D Kanamycin Agar, L-D Agar, L-D Esculin Agar, and L-D Bile Agar and most closely corresponds to CDC Presumpto Plate 1.

PRINCIPLE

Lombard-Dowell Agar Base supplemented with vitamin K and hemin supports the growth of common anaerobic bacteria. The cystine and sodium sulfite reduce the redox potential of the medium. Quadrant I contains kanamycin for differentiating kanamycin-resistant organisms from those which are kanamycin-susceptible. Quadrant II contains Lombard-Dowell Agar Base. It is used for detecting indole production, with tryptophan serving as a substrate. Quadrant III is used to detect esculin hydrolysis and catalase activity. Ferric ammonium citrate is added as the indicator of esculin hydrolysis. Quadrant IV contains 2% bile (oxgall), for detection of bile-resistant anaerobic gram-negative bacilli. This medium is useful in identifying *Bacteroides* and *Fusobacterium* spp.

REAGENTS (CLASSICAL FORMULAE)*

Base Medium:

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

pH 7.5 ± 0.2 @ 25°C

The following ingredients are added per liter of medium:

Quadrant I

Kanamycin	1.0 g

Quadrant III

Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g

Quadrant IV

Oxgall	20.0 g
Dextrose	1.0 g

*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. Place a sterile 1/4" blank disk on Quadrant II prior to incubation.
5. Incubate the Quad plate anaerobically for 48-72 hours at 33-37°C.
6. Following incubation, add 1-2 drops of Spot Indole Reagent (REF R21245) to the blank disk in Quadrant II. Observe for development of a blue color.
7. Add 1-2 drops of 3% hydrogen peroxide to a colony in Quadrant III and observe for bubbles.

INTERPRETATION OF THE TEST

Quadrant I (Kanamycin):
Positive Test - Growth (resistant)
Negative Test - No growth (sensitive)

Quadrant II (Indole):
Positive Test - Blue color development on disk
Negative Test - No color development on disk

Quadrant III (Esculin):
Positive Test - Black color development around colonies
Negative Test - No color change

Catalase:
Positive Test - Gas bubbles after addition of 3% hydrogen peroxide
Negative Test - No bubbles after addition of 3% hydrogen peroxide

Quadrant IV (Bile):
Positive Test - Growth equal to or greater than in Quadrant II (resistant)
Negative Test - Inhibition of growth or less growth than in Quadrant II (sensitive)

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QUALITY CONTROL

All lot numbers of Anaerobic Gram-Negative ID 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.

		RESULTS			
CONTROL	**INCUBATION**	**Kanamycin**	**Indole**	**Esculin**	**Bile**
Bacteroides ovatus ATCC® 8483	Anaerobic, 48-72 h @ 33-37°C	+	+	+	+
Bacteroides thetaiotaomicron ATCC® 29741	Anaerobic, 48-72 h @ 33-37°C	+	+	+	+
Fusobacterium mortiferum ATCC® 25557	Anaerobic, 48-72 h @ 33-37°C	-	-	+	+
Prevotella melaninogenica ATCC® 25845	Anaerobic, 48-72 h @ 33-37°C	+	-	-	-

LIMITATIONS

1. Use ρ-dimethylaminocinnamaldehyde indole reagent (Spot Indole Reagent, REF R21245) when performing the indole test in Quadrant II; do not use Ehrlich's or Kovacs' Indole Reagents.[3]

BIBLIOGRAPHY

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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. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

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.

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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