ANAEROBIC GRAM-POSITIVE ID QUAD

INTENDED USE
Remel Anaerobic Gram-Positive ID Quad is comprised of solid media recommended for use in qualitative procedures for the presumptive identification of anaerobic, gram-positive, sporeforming bacteria.

SUMMARY AND EXPLANATION
Three quadrant plates containing various differential media derived from Lombard-Dowell (L-D) Agar have been developed to aid in identifying anaerobic bacteria.[1] The quadrant plates have been designated by the Centers for Disease Control and Prevention (CDC) as Presumpto Plates 1, 2 and 3.[2] The Anaerobic Gram-Positive ID Quad corresponds to CDC Presumpto Plate 2 containing Glucose Agar, DNA Agar, Starch Agar, and Milk Agar. Story and Dowell recommended this quadrant plate for identification of *Clostridium* spp.[3]

PRINCIPLE
Lombard-Dowell Agar Base supplemented with the growth factors, 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 glucose for the detection of fermentation and growth stimulation. Brom thymol blue serves as the indicator of acid production during fermentation by a color change to yellow. Quadrant II contains DNA which is hydrolyzed by some obligately anaerobic bacteria. Toluidine blue is an indicator which results in a pink zone around growth when DNA is hydrolyzed. Quadrant III contains starch to test the ability of anaerobic bacteria to hydrolyze starch, resulting in a clearing of the medium when it is flooded with iodine. Quadrant IV contains milk proteins (casein) which may be hydrolyzed by anaerobic bacteria, resulting in a clearing of the medium.

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:

Glucose	6.0 g
Brom Thymol Blue (1% aqueous)	2.0 ml

Quadrant II:

Deoxyribonucleic Acid	1.25 g
Toluidine Blue (0.25% aqueous)	25.0 ml

Quadrant III:

Soluble Starch	5.0 g

Quadrant IV:

Skim Milk, powdered	50.0 g

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prepare an inoculum from a pure culture of the anaerobic test isolate using Thioglycollate Broth or Lombard-Dowell Broth equivalent to the density of a #1 McFarland Standard or equivalent (R20411).
2. Use a sterile Pasteur pipette to remove a portion of the bacterial suspension.
3. Add 1-2 drops to each quadrant and streak using a sterile loop.
4. Incubate Anaerobic Gram-Positive ID Quad anaerobically for 48-72 hours at 35-37°C.
5. Following incubation, flood quadrant III with iodine solution (e.g., Gram Iodine, R40056) and observe for clearing.

INTERPRETATION OF THE TEST

Quadrant I (Glucose Fermentation)
Positive Test - Yellow color development
Negative Test - No color change

Quadrant II (DNase)
Positive Test Pink zone around growth
Negative Test - No color change

Quadrant III (Starch Hydrolysis)
Positive Test - Clear zone around growth after addition of iodine
Negative Test - No zone around growth after addition of iodine

Quadrant IV (Milk Hydrolysis)
Positive Test - Clear zone around growth
Negative Test - No zone around growth

QUALITY CONTROL
All lot numbers of Anaerobic Gram-Positive 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.

CONTROL	INCUBATION	RESULTS			
		Glucose	DNase	Starch	Milk
Clostridium perfringens ATCC®13124	Anaerobic, up to 72 h @ 33-37°C	+	+	+	-
Clostridium sordellii ATCC® 9714	Anaerobic, up to 72 h @ 33-37°C	+	-	-	+
Clostridium tertium ATCC®19405	Anaerobic, up to 72 h @ 33-37°C	+	+	+	-
Veillonella parvula ATCC®10790	Anaerobic, up to 72 h @ 33-37°C	-	-	-	-

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BIBLIOGRAPHY

1. Dowell, V.R., Jr., G.L. Lombard, A.Y. Armfield, G.L. Jones, and T.M. Hawkins. 1981. Procedures for Use of Differential Agar Media in the Identification of Anaerobic Bacteria. U.S. Dept. of H.H.S. and Centers for Disease Control and Prevention, Atlanta, GA.
2. Dowell, V.R., Jr. and G.L. Lombard. 1977. Presumptive Identification of Anaerobic Nonsporeforming Gram-Negative Bacilli. U.S. Dept of Health, Education, and Welfare. Centers for Disease Control, Atlanta, GA.
3. Story, S. and V.R. Dowell, Jr. 1978. Development of a Presumpto Plate for Identification of Clostridia. Abstract C24. Annual Meeting of the American Society for Microbiology. Las Vegas, NV.

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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