HAEMOPHILUS ID QUAD

INTENDED USE
Remel Haemophilus ID Quad is comprised of solid media recommended for use in qualitative procedures to speciate the genus *Haemophilus* based on demonstration of growth factor requirements and production of hemolysis on horse blood.

SUMMARY AND EXPLANATION
Haemophilus spp. are small, gram-negative bacilli that require the growth factors hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD or V factor) for growth.[1] In 1921, Davis determined X and V factors were associated with the satellite phenomenon observed with *Haemophilus* spp.[2] Thjotta and Avery reported X factor is derived from hemoglobin and V factor from the satellite relationship with *Staphylococcus aureus*.[3] Lwoff and Lwoff later identified V factor as nicotinamide adenine dinucleotide (NAD).[4] The need for one or both growth factors along with hemolytic reactions on horse blood agar provides a reliable means of identifying *Haemophilus* spp.[5,6]

PRINCIPLE
Haemophilus ID Quad contains a nutritious beef heart infusion supplemented with growth factors. Quadrant I is supplemented with hemin (X factor), Quadrant II is supplemented with NAD (V factor), and Quadrant III is supplemented with both hemin and NAD. Quadrant IV, also supplemented with NAD, contains horse blood (also a source of hemin) and provides for the demonstration of hemolytic reactions by *Haemophilus* spp. The growth factor requirements are demonstrated by growth or no growth of the test isolate in each quadrant.

REAGENTS (CLASSICAL FORMULAE)*
Base Medium:

Casein Peptone	13.0 g	Beef Heart Infusion	2.0 g
Sodium Chloride	5.0 g	Agar	15.0 g
Yeast Extract	5.0 g	Demineralized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

The following ingredients are added per liter of medium:

Hemin (Quadrants I and III)	20.0 mg	NAD (Quadrants II, III, and IV)	0.1 g
Horse Blood (Quadrant IV)	50.0 ml		

*Adjusted as required to meet performance standards.

PROCEDURE
1. Select one or two well-isolated colonies from the primary isolation medium that resemble *Haemophilus* spp., both by Gram stain and colony morphology.
2. Suspend colonies in 5 ml of Tryptic Soy Broth (REF R07224) or sterile physiological saline. (Do NOT cool the inoculating loop in the primary isolation medium before selecting colonies due to the possibility of carryover of growth factors.) Vortex to mix the suspension.
3. Inoculate each Quadrant with 1 loopful of the suspension and streak for isolation. To prevent carryover of growth factors, flame the loop between each Quadrant.
4. Incubate plate in 5-10% CO_2 at 33-37°C for 18-24 hours.
5. Examine plates for growth or no growth in Quadrants I, II, and III, and a hemolytic reaction in Quadrant IV.

INTERPRETATION OF THE TEST
Quadrants I, II, II:
Positive Test - Growth
Negative Test - No growth

Quadrant IV:
Positive Test - Growth w/ β-hemolysis
Negative Test - Growth w/ no hemolysis

QUALITY CONTROL
All lot numbers of Haemophilus 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 ORGANISM	INCUBATION	RESULTS			
		I – X factor	II – V factor	III–X&V factor	IV–V factor, Horse Bld.
Haemophilus aphrophilus ATCC® 19415	CO_2, 18-24 h @ 33-37°C	Growth	Growth	Growth	Growth
Haemophilus influenzae ATCC® 10211	CO_2, 18-24 h @ 33-37°C	No growth	No growth	Growth	Growth
Haemophilus influenzae ATCC® 49247	CO_2, 18-24 h @ 33-37°C	No growth	No growth	Growth	Growth
Haemophilus parahaemolyticus ATCC® 10014	CO_2, 18-24 h @ 33-37°C	No growth	Growth	Growth	Growth, β-hemolysis
Haemophilus parainfluenzae ATCC® 7901	CO_2, 18-24 h @ 33-37°C	No growth	Growth	Growth	Growth

(Continued on back)

LIMITATIONS

1. Clinical specimens may contain more than one species of *Haemophilus*; exercise strict attention to colony morphology and hemolytic reaction when selecting colonies from primary isolation media.[6]
2. The use of excessive inoculum may result in erroneous identifications. Some strains of *H. influenzae* may appear to be X factor-independent (i.e., growth in Quadrant II) due to traces of hemin compounds carried over in a heavy inoculum.[6]
3. Compare the growth in Quadrant II to the growth in Quadrant III, as well as any growth in Quadrants I and II with that of III and IV. The level or amount of growth in each Quadrant should be comparable. Noticeable variation in levels of growth may indicate carryover of factors from the inoculum or primary isolation medium, and result in false identification of the test isolate.[6]

BIBLIOGRAPHY

1. Winn, W., S. Allen, J. William, E. Koneman, G. Procop, P. Schreckenberger, and G. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins, Baltimore, MD.
2. Davis, D.J. 1921. J. Infect. Dis. 29:178.
3. Thjotta, T. and O.T. Avery. 1921. J. Exp. Med. 34:97-114.
4. Lwoff, A. and M. Lwoff. 1937. Proc. R. Soc. (London). 122:352
5. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.
6. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.

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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