



QUALITY CONTROL PROCEDURES

I INTRODUCTION

The Hemo (Haemophilus) ID QUAD plate is for the identification of *Haemophilus* species.

II PERFORMANCE TEST PROCEDURE

1. Streak-inoculate representative samples with the cultures listed below.
2. Use 18- to 24-h broth cultures diluted 10⁻¹.
3. Incubate plates at 35 ± 2°C in an aerobic atmosphere supplemented with carbon dioxide.
4. Examine plates after 18–24 h for growth of *Haemophilus*.
5. Expected Results

Organisms	ATCC™	Growth on Quadrant			Hemolysis
		I	II	III	IV
* <i>Haemophilus influenzae</i>	10211	–	–	+	–
* <i>Haemophilus parahemolyticus</i>	10014	–	+	+	+

To test quadrant I for the presence of X factor, inoculate with *H. influenzae* ATCC 10211 and add a V factor strip or disc to the center of the quadrant. Growth will occur if X factor is present in the medium.

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Note the firmness of plates during the inoculation procedure.
4. Incubate uninoculated representative plates at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

The Hemo (Haemophilus) Identification (ID) QUAD Plate (with growth factors) is used for the differentiation and identification of *Haemophilus* species based on requirements for growth factors X and/or V and hemolytic reactions.

V SUMMARY AND EXPLANATION

In 1917, Davis reported that influenza bacteria required two factors for *in vitro* growth, one from hemoglobin and another that the organism derived through a satellite relationship with *Staphylococcus aureus*.¹ In 1921, Thjotta and Avery described the growth factor from blood as X factor and the other as V factor.² The X factor was found in hemin and later identified as protoporphyrin IX, or protoheme, and V factor was identified as nicotinamide adenine dinucleotide (NAD).^{3,4}

Pathogenic *Haemophilus* species may be differentiated and presumptively identified by determining *in vitro* growth requirements for X and/or V factors and by determining hemolytic reactions.

VI PRINCIPLES OF THE PROCEDURE

The Hemo ID QUAD Plate (with growth factors) is a four-sectored plate that contains Brain Heart Infusion Agar in quadrants I, II and III and Blood Agar Base in quadrant IV. Quadrant I is enriched with hemin to supply X factor. Quadrant II is enriched with IsoVitaleX™ Enrichment to supply V factor and other nutrients, such as thiamine and cysteine, which stimulate the growth of *Haemophilus* species. Quadrant III contains both hemin and IsoVitaleX Enrichment. Quadrant IV is supplemented with NAD to supply V factor and horse blood to provide X factor and demonstrate hemolytic reactions.

A *Haemophilus* isolate that grows on quadrants III and IV, but fails to grow on quadrants I and II exhibits a requirement for both X and V factors. An isolate that fails to grow on quadrant I, but grows on quadrants II, III and IV indicates a requirement only for V factor. Alternatively, an isolate that fails to grow on quadrant II, but grows on quadrants I, III and IV indicates a requirement only for X factor.

VII REAGENTS

Brain Heart Infusion Agar (BHIA)

Approximate Formula* Per Liter Purified Water

Brain Heart, Infusion from (solids)	8.0 g
Peptic Digest of Animal Tissue.....	5.0 g
Pancreatic Digest of Casein	16.0 g
Sodium Chloride	5.0 g
Dextrose	2.0 g
Disodium Phosphate.....	2.5 g
Agar	13.5 g

Blood Agar Base (BAB)

Approximate Formula* Per Liter Purified Water

Heart Muscle, Infusion from (solids)	2.0 g
Pancreatic Digest of Casein	13.0 g
Yeast Extract	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

IsoVitaleX Enrichment

Approximate Formula* Per Liter Purified Water

Vitamin B ₁₂	0.01 g	Thiamine Pyrophosphate	0.1 g
L-Glutamine	10.0 g	Ferric Nitrate	0.02 g
Adenine.....	1.0 g	Thiamine Hydrochloride	0.003 g
Guanine Hydrochloride	0.03 g	L-Cysteine Hydrochloride	25.9 g
p-Aminobenzoic Acid.....	0.013 g	L-Cystine.....	1.1 g
Nicotinamide Adenine Dinucleotide.....	0.25 g	Dextrose.....	100.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

Quadrant I consists of BHIA with approximately 20.0 mg/L hemin.

Quadrant II consists of BHIA with approximately 10.0 mL/L IsoVitaleX Enrichment.

Quadrant III consists of BHIA with approximately 20.0 mg/L hemin and 10.0 mL/L IsoVitaleX Enrichment.

Quadrant IV consists of BAB with approximately 10 mL/L IsoVitaleX Enrichment and 5% horse blood.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

A variety of swabs and containers have been devised for collecting specimens. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory. Several holding media or transport systems, such as BBL specimen collection and transport products, have been devised to prolong the survival of microorganisms when a significant delay is expected between collection and definitive culturing.

Refer to appropriate texts for details of specimen collection and handling procedures.^{5,6}

IX PROCEDURE

Material Provided: Hemo ID QUAD (with Growth Factors)

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: The agar surface should be smooth and moist, but without excessive moisture.

The initial specimens should be inoculated onto BBL Chocolate II Agar or another suitable medium and incubated for 18–24 h in a CO₂-enriched atmosphere. Choose one or two colonies that resemble *Haemophilus* species and perform a Gram stain to confirm that the isolate is a gram-negative rod or coccobacillus.

To prepare inoculum, suspend several well-isolated colonies of the test organism from an 18–24 h plate culture into 5 mL of distilled water or Trypticase™ Soy Broth or other suitable medium and adjust the turbidity to a 0.5 McFarland turbidity standard. Dilute 10⁻¹. Inoculate each quadrant with one loopful of the diluted specimen and streak to obtain isolated colonies. To prevent carry-over of growth factors, sterilize the loop between inoculations of each quadrant.

Alternatively, suspend one or two colonies in 5 mL of distilled water or Trypticase Soy Broth or other suitable medium and vortex to mix. Inoculate each quadrant of the plate with one loopful of the diluted specimen and streak to obtain isolated colonies, sterilizing the loop between inoculations of each quadrant.

Do not inoculate plates directly from the chocolate plate. Inoculum must be diluted as described above.

Invert the plates (agar side up) and incubate them in a CO₂-enriched atmosphere at 35°C for 24 h and examine for growth.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After 24 h of incubation, the plates should show growth or no growth, depending on X and V factor requirements. The isolate should grow in quadrants III and IV. In quadrant IV, the isolated colonies may have zones of hemolysis, depending on the species isolated.

The following table shows the expected growth results for various *Haemophilus* species:

	Quadrants (Factors Present)			
	I(X)	II(V)	III(XV)	IV Hemolysis
<i>H. influenzae</i>	–	–	+	–
<i>H. haemolyticus</i>	–	–	+	+
<i>H. parainfluenzae</i>	–	+	+	–
<i>H. parahaemolyticus</i>	–	+	+	+

Gram-staining, biochemical tests and/or additional identification procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

These plates are only intended to be used as part of the scheme of identification of *Haemophilus* species. Consult appropriate references for information about Gram-staining, biochemical tests and other procedures.⁷⁻⁹

Carryover of hemin (X factor) from chocolate agar may occur when transferring a heavy inoculum or direct growth to the Hemo ID QUAD plate. This could result in incorrect interpretation of growth factor requirements and possible organism misidentification. By preparing a suspension of growth comparable to a 0.5 McFarland turbidity standard and then diluting the suspension 10⁻¹, the growth factor requirements can be accurately determined. Trace growth in quadrants I and II, when compared to growth in quadrants III and IV, should be considered as "no growth." Compare growth in quadrants I and II with growth in quadrants III and IV. If comparable or a greater amount of growth, consider the result as positive.

If heavy growth appears on all four quadrants, prepare a Gram stain to determine purity and that morphological characteristics are appropriate for *Haemophilus*.

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Hemo (Haemophilus) ID QUAD with Growth Factors are tested for performance characteristics. Representative samples of the lot are tested with *Haemophilus parahaemolyticus* ATCC 10014 and *H. influenzae* ATCC 10211, inoculated using normal saline suspensions diluted to yield 1 x 10³ to 1 x 10⁴ CFU/quadrant. A V factor strip is placed on quadrant I inoculated with *H. influenzae* to test for presence of X factor. Plates are incubated at 35–37°C for one day in an aerobic atmosphere. *H. parahaemolyticus* grows on quadrants II, III and IV containing the V factor. *H. influenzae* grows on quadrants I, III and IV which have both X and V factors. Beta hemolysis is observed on quadrant IV for *H. parahaemolyticus*.

XIII AVAILABILITY

Cat. No.	Description
297890	BBL™ Hemo ID QUAD (With Growth Factors), Pkg. of 10 plates

XIV REFERENCES

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