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HARDY DIAGNOSTICS
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Instructions for Use

HAEMOPHILUS ID QUADPLATE

Cat. no. J82	Haemophilus ID Quadplate, 15x100mm Quadplate, 5ml/quadrant	10 plates/bag

INTENDED USE

The Hardy Diagnostics Haemophilus ID Quadplate is recommended for the differentiation of *Haemophilus* species.

SUMMARY

Members of the genus *Haemophilus* are differentiated by requirements for X- and V-factors and hemolytic reactions on either rabbit blood, horse blood or RTF Medium with sheep blood. RTF Medium allows *Haemophilus* to grow and show appropriate hemolytic patterns with Sheep Blood. The Haemophilus ID Quadplate consists of four separate formulations, which supply the requirements as follows:

RTF Casman Medium, Modified	X- and V-factors
Tryptic Soy Agar (TSA) with Hemin	X-factor
Tryptic Soy Agar (TSA), Modified with NAD	V-factor
Chocolate Agar	X- and V-factors

FORMULA

Viewing from the top of the plate, in clockwise order beginning with the red quadrant, the media formulas per liter of deionized water are as follows:*

Quadrant I:	
RTF Casman Medium, Modified	43.0gm
Sheep Blood	50.0ml
Quadrant II:	
Tryptic Soy Agar (TSA)	40.0gm
Hemin (X-factor)	0.02gm
Quadrant III:	
Tryptic Soy Agar (TSA), Modified	40.0gm
NAD (V-factor)	0.10gm
Quadrant IV:	

GC Agar Base	36.0gm
Hemoglobin	10.0gm
Koenzyme (X- and V-factors)	10.0ml

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organisms. This product is used in conjunction with other biochemical tests to identify cultures of isolated organisms.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Select one or two well isolated colonies that resemble *Haemophilus* species both by gram stain and morphology and dilute in 5ml of sterile Tryptic Soy Broth (Cat. no. R30) or sterile Saline, 0.85% (Cat. no. K59). Vortex to mix. Do not inoculate directly from the colony to the quadplate. Due to the possibility of carryover of growth factors, **do not** cool the inoculating loop in the primary isolation medium before selecting colonies. **Do** flame the loop between inoculation of each quadrant. Be

careful not to pick up any culture media on the loop when picking colonies for broth inoculation. Incubate the plate in 5-10% CO_2 at 35-37ºC. for 18-24 hours. Examine plates for growth, no growth and hemolytic reactions.

INTERPRETATION OF RESULTS

Quadrants are identified by starting with the red quadrant and proceeding in clockwise order.

Disregard very light growth in quadrants containing TSA with Hemin (quadrant 2) and TSA, Modified with NAD (quadrant 3) compared to the growth of the RTF Casman, Modified (quadrant 1) and Chocolate Agar (quadrant 4).

Growth in quadrants 2 and 3 must be as great as quadrant 4 in order to be called positive; i.e., slight growth is interpreted as a negative.

Organisms	**RTF Casman, Modified** (Quadrant 1)		**X-Factor** TSA with Hemin (Quadrant 2)	**V-Factor** TSA, Modified with NAD (Quadrant 3)	**X- and V-Factors** Chocolate Agar (Quadrant 4)
	Growth	Hemolysis			
H. influenzae	+	-	-	-	+
H. haemolyticus	+	+	-	-	+
H. parainfluenzae	+	-	-	+	+
H. parahaemolyticus	+	+	-	+	+
H. aphrophilus *	+	-	V	-	+
*H. paraphrophilus**	+	-	-	+	+
H. aegyptius	+	-	-	-	+
H. segnis *	+	-	-	+	+

KEY: + = Growth or hemolysis - = No growth V = Variable

* *H. aphrophilus* and *H. paraphrophilus* were recently reclassified as *Aggregatibacter aphrophilus* and *H. segnis* was recently reclassified as *Aggregatibacter segnis* .

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Clinical specimens may contain more than one species of *Haemophilus* ; therefore, strict attention to colony morphology and hemolytic reaction is necessary when selecting colonies from primary isolation media.

Avoid excess inoculum. Refer to " Inoculation Procedures " on the Hardy Diagnostics website for a description of the proper inoculation method. Some strains of *Haemophilus influenzae* may appear to have no X-factor requirement due to traces of hemin compounds carried over from a heavy inoculum. **Care should be taken during inoculation of specimens onto culture media in order to prevent nutrient carryover.**

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, V-Factor Disk (Cat. no. Z7041), swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not

provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Haemophilus influenzae ATCC® 10211	B	24hr	35°C	CO_2**	**RTF Casman, Modified:** Growth with no hemolysis **TSA with Hemin:*** ** Growth only around the V-Factor Disk **TSA, Modified with NAD:** No growth **Chocolate Agar:** Growth
Haemophilus parahaemolyticus ATCC® 10014	B	24hr	35°C	CO_2 **	**RTF Casman, Modified:** Growth with hemolysis **TSA with Hemin:** No growth **TSA , Modified with NAD:** Growth **Chocolate Agar:** Growth

Note: When interpreting results, growth in quadrants 2 and 3 must be as great as quadrant 4 in order to be called positive; i.e., slight growth is interpreted as negative. Always prepare a suspension of the organisms before inoculation. When inoculating, the loop must be flamed between streaking of each quadrant.

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% CO_2.

*** For QC purposes, after streaking the test organism on the plate, a V-Factor Disk (Cat. no. Z7041) should be placed on the center of the quadrant.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

- RTF Casman, Modified should appear opaque, and cherry red in color.
- TSA with Hemin should appear slightly opalescent, and light amber in color.
- TSA, Modified with NAD should appear slightly opalescent, and light beige in color.
- Chocolate Agar should appear opaque, and brown in color.

Haemophilus ID Quad Plate (Cat. no. J82).
Showing growth of *Haemophilus influenzae* (ATCC® 10211) colonies on RTF Casman, Modified (no hemolysis) and Chocolate Agar sectors. No growth on TSA with Hemin or TSA, Modified with NAD sectors. This growth pattern was indicative of an organism that requires both X-factor and V-factor for growth. This was consistant with *H. influenzae* . Incubated in CO_2 for 48 hours at 35ºC.

Haemophilus ID Quad Plate (Cat. no. J82).
Showing growth of *Haemophilus parahaemolyticus* (ATCC® 10014) colonies on RTF Casman, Modified (with hemolysis); TSA, Modified with NAD and Chocolate Agar sectors. No growth on TSA with Hemin sector. This growth pattern was indicative of an organism that does not require X-factor for growth but does require V-factor. This was consistant with *H. parahaemolyticus* . Incubated in CO_2 for 48 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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