IFU

Instructions for Use

X- AND V-FACTOR DISKS

Cat. no. Z7031	X-Factor	1x50 disks/cartridge
Cat. no. Z7041	V-Factor	1x50 disks/cartridge
Cat. no. Z7051	XV-Factor	1x50 disks/cartridge

INTENDED USE

Hardy Diagnostics X - and V-Factor Disks are paper disks impregnated with X (hemin) and V (nicotinamide adenine dinucleotide - NAD) growth factors. They are used for the differentiation of *Haemophilus* species, including *Aggregatibacter aphrophilus* based upon their requirements for the growth factors.

SUMMARY

Haemophilus spp. have varying requirements for X and V growth factors. Consequently, the significant differences in growth factor requirements of *Haemophilus* spp. allows for their differentiation. Differentiation is based on the presence or absence of growth around and/or between disks impregnated with factors X, V and XV.

Use of paper disks impregnated with the growth factors was first proposed by Parker and Hoeprich.[4]

The determination that *H. influenzae* requires two growth factors was made by Davis, Thjotta, and Avery.[1,2]

FORMULA

Each X-Factor Disk is impregnated with hemin. Each V-Factor Disk is impregnated with NAD (nicotinamide adenine dinucleotide). Each XV-Factor Disk is impregnated with a combination of hemin and NAD.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at -20 to 8ºC. away from direct light. The disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

The expiration date applies to the product in its intact packaging when stored as directed.

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

1. Using aseptic techniques, select isolated colonies of suspect colonies and prepare a suspension equivalent to a 0.5 McFarland standard (Cat. no. MS05). Tryptic Soy Broth (Cat. no. R30) or sterile Saline, 0.85% (Cat. no. K59) can be used for making suspensions.

Note: Due to the possibility of carryover of growth factors, **do not** cool the inoculating loop in the primary isolation medium before selecting colonies.

2. Using a sterile swab, inoculate the entire surface of a factor-free media such as Brain Heart Infusion Agar (Cat. no. W15). If Brain Heart Infusion Agar is unavailable, Mueller Hinton Agar (Cat. no. G45) or Tryptic Soy Agar (Cat. no. G60) may be substituted. Inoculate by streaking in three different directions by rotating the plate in a 60 degree angle after each streaking. Streaking in this manner ensures confluent growth.

3. Allow the surface to dry 3-5 minutes.

4. Using aseptic technique, apply disks around the periphery of the plate (approximately 1-2cm from the edge of the medium). Apply disks in the following positions:

X-Factor Disk	12 o'clock
V-Factor Disk	4 o'clock
XV-Factor Disk	8 o'clock

5. Incubate inoculated media 18-48 hours at 35ºC. in 5-10% CO_2. Observe for growth or no growth around the disks.

INTERPRETATION OF RESULTS

Organisms that require only X-Factor will grow only in the area of the X- and XV-Factor Disks. Organisms that require only V-Factor, will grow only in the areas of the V- and the XV-Factor Disks. If both X- and V-Factors are required, the organism will grow only in the area of the XV-Factor Disk.

In cases where slight growth occurs around the V-Factor Disk, the growth must be at least equal in amount to that around the XV-Factor Disk before the organism can be considered *H. parainfluenzae*.

***Haemophilus* species**	**Growth Around Disk**		
	X	**V**	**XV**

Haemophilus aegyptius	-	-	+
Haemophilus aphrophilus	v/-	-	+
Haemophilus ducreyi	+	-	+
Haemophilus haemolyticus	-	-	+
Haemophilus influenzae	-	-	+
Haemophilus parahaemolyticus	-	+	+
Haemophilus parainfluenzae	-	+	+
Aggregatibacter aphrophilus (formerly *H. parainfluenzae*, ATCC® 7901)	-	+	+

LIMITATIONS OF THE PROCEDURE

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.

Because similarities exist in growth factor requirements of *Haemophilus* species, it is not recommended that this procedure be the sole criterion for species identification.

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, slides, staining supplies, other culture media, microscopes, incinerators, and incubators, etc., as well as catalase, and other biochemical and serological reagents, are not provided.

QUALITY CONTROL

Expected Results	
Test Organisms	**Reaction**
Haemophilus influenzae ATCC® 10211	X-Factor: No Growth V-Factor: No Growth XV-Factor: Growth
Aggregatibacter aphrophilus (formerly *Haemophilus parainfluenzae)* ATCC® 7901	X-Factor: No Growth V-Factor: Growth XV-Factor: Growth

USER QUALITY CONTROL

It is recommended that each lot or shipment of XV-Factor disks be checked with a positive control, and the X-Factor and V-Factor disks be tested with both known positive and negative controls.[6,7]

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.

Showing growth of *Haemophilus influenzae* (ATCC® 10211) around the XV-Factor Disk (Cat. no. Z1051) only. This growth pattern demonstrated the inability of the organism to grow in the absense of either V-Factor or X-Factor and was consistant with *H. influenzae*. Incubated on Mueller Hinton Agar (Cat. no. G45) in CO_2 for 48 hours at 35ºC.

Showing closer image of *Haemophilus influenzae* growth around the XV-Factor disk from the photo to the left.

REFERENCES

1. Davis, D.J. 1917. *J. Infect. Dis.*; 21:392.

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3. Lwoff, A., and Lwoff, M. 1937. Studies on codehydrogenosis, *I. Proc. Roy. Soc. London Ser. B.*; 122:352-359.

4. Parker, R.H., and Hoeprich, P.D. 1962. *Am. J. Clin. Path.*; 37:319-327.

5. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

6. *Commission on Laboratory Accreditation, Laboratory Accreditation Program Microbiology Checklist*. College of American Pathologists. Rev. 9/30/2004.

7. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services*. Subpart K - Quality System for Non-Waived Testing. 493;1200-1265. www.cms.hhs.gov/clia.

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