IFU

Instructions for Use

ALA DIFFERENTIATION DISKS

Cat. no. Z7081	ALA Differentiation Disks	50 disks/cartridge

INTENDED USE

HardyDisk™ ALA (delta-aminolevulinic) Differentiation Disks rapidly detect the presence of porphyrin and cytochrome compounds and are used to differentiate *Haemophilus* species, including *Aggregatibacter aphrophilus*.

SUMMARY

Traditionally *Haemophilus* species have been differentiated by their varying requirements for hemin (X-Factor), NAD (nicotinamide adenine dinucleotide, V-Factor), and a combination of hemin and NAD (XV-Factor). However, erroneous results have been demonstrated when using growth factor requirement tests. These misidentifications are largely attributed to the carryover of X-Factor in the inoculum as well as the presence of trace amounts of X-Factor in the medium. [(5)] The use of HardyDisk™ ALA Differentiation Disk is used as an alternative method to X-Factor requirement testing. The ALA procedure is a more rapid test method as well as a more accurate method for determining hemin requirements by eliminating the erroneous results associated with X-Factor requirement tests.[(8)]

HardyDisk™ ALA Differentiation Disk assesses the ability of a *Haemophilus* strain to synthesize hemin from the ALA substrate. The test detects the presence of porphyrin compounds, which are intermediates in the hemin biosynthetic pathway.[(4,5)] Porphyrins are detected by the emission of a red-orange fluorescence under UV light (366nm) and indicate that the organism is capable of synthesizing hemin and is not dependent on hemin (X-Factor) for growth. Conversely, hemin requiring strains lack the enzymes to synthesize hemin and do not produce intermediate porphyrin compounds.

FORMULA

Each HardyDisk™ ALA Differentiation Disk is prepared by impregnating carefully controlled concentrations of delta-aminolevulinic acid onto a high quality 6mm diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8ºC. away from direct light. Disks should not be used if there are any signs of discoloration 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: 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 organism.

Method of Use:

1. Perform HardyDisk™ ALA Differentiation Disks only on isolates either growing on Chocolate Agar in 18-24 hours or that satellite around *Staphylococcus aureus* on a blood agar plate. See "Precautions" section above.

2. Prior to use, allow the disks to equilibrate to room temperature.

3. Aseptically place the disk into a sterile petri plate. Wet the disk with a small drop (0.04ml) of sterile saline (Cat. no. R45).

4. Inoculate disk with several well isolated 18-24 hour colonies to yield a visible cell paste on the disk surface. Alternatively, the disk can be touched to a colony and then placed in a petri dish.

5. Moisten a piece of filter paper with water and place it into the lid of the petri plate to ensure that the disk is kept moist during incubation.

6. Incubate the disks aerobically at 35ºC. for up to 2 hours.

7. After 2 hours, examine the disk under ultra-violet light (366nm) in a darkened room. Observe the disk for the presence of a red fluorescence while under UV light.

INTERPRETATION OF RESULTS

A positive reaction is recorded when orange/red fluorescence is observed on the HardyDisk™ ALA Differentiation Disk. This color change is a positive result for porphyrin synthesis and indicates that the organism does not require hemin (X-Factor) for growth.

A negative result is recorded when no fluorescence is observed on the disk and indicates that porphyrin was not synthesized. Consequently, the organism requires hemin for growth.

***Haemophilus* species**	**Growth Factor Requirements**		**Porphyrin Synthesis**

	X	V	ALA
Haemophilus ducreyi	+	-	-
Haemophilus haemolyticus	+	+	-
Haemophilus influenzae	+	+	-
Haemophilus parahaemolyticus	-	+	+
Haemophilus parainfluenzae	-	+	+
Aggregatibacter aphrophilus (formerly *H. aphrophilus and H. paraphrophilus*)	-	V	+

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.

In order to avoid inaccurate results it is recommended that organisms be less than 24 hours old, a heavy inoculum is used to inoculate the disk, and that the disk is kept moist during incubation.(4)

H. duceryi may not be identified using this procedure as some strains do not grow as satellite colonies on blood agar and grow more slowly, on the magnitude of several days, on enriched media.

Because similarities exist in growth factor requirements of *Haemophilus* species, it is not recommended that this procedure be the sole criterion for species identification.(4,5) Consult listed references for additional information regarding the recommended tests for complete identification of *Haemophilus* species.(2-5)

The ALA test, even in conjunction with the satellite test, does not differentiate *H. influenzae* and *H. haemolyticus*. *H. haemolyticus* is not considered pathogenic and is separated from *H. influenzae* by a positive hemolysis reaction on rabbit or horse blood agar.

Francisella spp., including *Francisella tularensis*, is ALA negative and grows only on chocolate agar. It can be differentiated from *H. influenzae* as it grows more slowly, does not satellite on blood agar, and is not V-Factor dependent.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as UV light, loops, other culture media, 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	
Aggregatibacter aphrophilus (formerly *H. parainfluenzae*)	*	2hr	35°C	Aerobic	Positive for porphyrin synthesis; orange/red fluorescence in the presence of

ATCC® 7901					UV light
Haemophilus influenzae ATCC® 10211	*	2hr	35°C	Aerobic	Negative for porphyrin synthesis; no red fluorescence in the presence of UV light

*Refer to the procedure outlined above.

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

HardyDisk™ ALA Differentiation Disks are 6mm (in diameter) filter paper disks with the letters ALA printed on both sides, and should appear white in color.

Showing positive (left disk) and negative (right disk) HardyDisk™ ALA Differentiation Disks (Cat. no. Z7081) under UV light.
LEFT DISK: *Aggregatibacter aphrophilus* (ATCC® 7901) growth applied to ALA Disk. The orange/red color under UV light was indicative as **positive.**
RIGHT DISK: *Haemophilus influenzae* (ATCC® 10211) growth applied to ALA Disk. No orange/red color under UV light was indicative as **negative.**

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.

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

4. Howard, B.J., et al. 1987. *Clinical and Pathogenic Microbiology* , 2nd ed. C.V. Mosby Company, St. Louis, MO.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

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7. Wong, J.D. 1987. Porphyrin test as an alternative to benzidine test for detecting cytochromes in catalase-negative, gram-positive cocci. *J. Clin. Micriobiol.*; 25:2006-2007.

8. Lund M.S. and D.J. Blazevic. 1977. Rapid speciation of *Haemophilus* with the porphyrin production test vs. the satellite test for X. *J. Clin. Microbiol.*; 5:142-144.

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

HDQA 2207F [D]