IFU

Instructions for Use

HardyDisk™ BILE DIFFERENTIATION DISKS

Cat. no. Z7091	Bile Differentiation Disks	50 disks/cartridge

INTENDED USE

HardyDisk™ Bile Differentiation Disks are used for the differentiation of *Bacteroides fragilis* group and for identification of other gram-negative anaerobic bacilli.

SUMMARY

Anaerobic gram-negative bacilli are the most commonly encountered anaerobes in clinical specimens, with *Bacteroides fragilis* group isolated more frequently than any other anaerobe.[4] Organisms in the *Bacteroides* genus have received notoriety due to their frequent involvement in infectious disease and their resistance to antimicrobial agents. Penicillin-resistant strains of the *B. fragilis* group are common, however, there are recent reports of new resistance to cefotetan and clindamycin and occasional resistance to piperacillin-tazobactam, imipenem and quinolones.[6] HardyDisk™ Bile Differentiation Disks are instrumental in differentiating the *B. fragilis* group from other*Bacteroides* and *Prevotella*species.[2,6]

The HardyDisk™ Bile Differentiation Disk is used to determine an organism's ability to grow in the presence of high concentrations of bile. Other indicators of bile resistance, including significant growth on BBE (Bacteroides Bile Esculin) media and growth in 20% bile broth, require the inoculation of additional media. In 1983, it was demonstrated that comparable results were obtained using a 15mg bile disk and traditional bile tolerance methods. This study confirmed that the bile disk method is capable of clearly distinguishing between bile-resistant and bile-sensitive anaerobic organisms.[2] A bile disk is a rapid and cost effective method for detecting bile resistance. The disk can be conveniently added to a Brucella subculture plate when a gram stain reveals an isolate to be an anaerobic gram-negative rod.

HardyDisk™ Bile Differentiation Disks can be used to determine bile sensitivity in a variety of organisms. An anaerobic, gram-negative rod that is bile-tolerant and resistant to vancomycin, kanamycin, and colistin can be identified as a member of the *Bacteroides fragilis* group. Additionally, *Bilophila* which are phenotypically similar to *B. ureolyticus*, can be differentiated by bile tolerance and a strong catalase reaction. Bile resistance is also useful in the presumptive differentiation of *Fusobacterium mortiferum* and *Fusobacterium varium* from other *Fusobacterium* species.[2,6]

FORMULA

Each HardyDisk™ Bile Differentiation Disk is prepared by impregnating carefully controlled concentrations of bile 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. Product should not be used if there are any signs of

deterioration or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, and moisture.

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. Allow disks to equilibrate to room temperature before use. Prepare a suspension, equivalent to a 0.5 McFarland opacity standard, of the organism to be tested in Thioglycollate Broth (Cat. no. K21).

2. Dip a sterile non-toxic swab (Cat. no. Z5800R) the organism suspension. Rotate the swab several times, pressing firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. Evenly inoculate the dried surface of Brucella Agar with Hemin and Vitamin K (Cat. no. A30) to obtain heavy confluent growth.

3. Aseptically place a single bile disk on the media surface. With sterile forceps, gently tap each disk to the media surface to ensure uniform diffusion of the bile into the medium.

4. Incubate anaerobically at 35ºC. for 24-48 hours.

5. When adequate growth is present, examine the plate for a zone of inhibition.

INTERPRETATION OF RESULTS

An organism is considered sensitive to bile when a zone of inhibition is present around the disk

An organism is considered resistant to bile when there is no zone of inhibition surrounding the disk.

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.

The presence of bile disks will cause a zone of hemolysis on blood based media, however, this is not an indication of organism growth.

Among the *Bacteroides fragilis* group, some *B. uniformis* strains may grow poorly in the presence of bile and will have a zone of inhibition around the disk.(6)

Some non- *B. fragilis* group are bile-resistant; morphology, biochemical tests and other special potency disks will differentiate these species from the *B. fragilis* group.

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 (including Thioglycollate Broth and Brucella Agar with H and K), 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	
Bacteroides fragilis ATCC® 25285	F	24-48hr	35 ° C	Anaerobic	Resistant; no zone of inhibition
Prevotella melaninogenica ATCC® 25845	F	24-48hr	35° C	Anaerobic	Sensitive; zone of inhibition present

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

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™ Bile Differentiation Disks are 6mm (in diameter) filter paper disks with the letters BILE printed on both sides and should appear beige in color.

Bile-Resistant (no zone of inhibition)
Bacteriodes fragilis (ATCC® 25285) growing around a HardyDisk™ Bile Differentiation Disk (Cat. no. Z7901) on Brucella Agar with Hemin and Vitamin K (Cat. no. A30). Incubated anaerobically for 48 hours at 35ºC.

Bile-Sensitive
Prevotella melaninogencia (ATCC® 25845) growing with a zone of inhibition around a HardyDisk™ Bile Differentiation Disk (Cat. no. Z7901) on Brucella Agar with Hemin and Vitamin K (Cat. no. A30). Incubated anaerobically for 48 hours at 35 deg. C.

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

6. Jousimies-Somer, H., et al. 2002. *Wadsworth Anaerobic Bacteriology Manual*, 6th ed. Star Publishing, Belmont, CA.

IFU-10471[A]

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207F [D]