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 +8 degrees C. 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 applies to the product in its intact packaging when stored as directed.

Refer to the keyword "Storage", in the Hardy Diagnostics software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers of Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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.

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics software program HUGO™, for more information on handling potentially hazardous material.

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 35 degrees C. 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 and/or serological tests be performed on colonies from pure culture for complete identification.

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 keyword "Limitations", in the Hardy Diagnostics software program HUGO™, for more information regarding general limitations on culture media.

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

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Time	Incubation Temperature	Atmosphere	Results
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

User Quality Control

Check for signs of contamination and deterioration. It is recommended that each new lot of disks be tested with known positive and negative controls.

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics software program HUGO™, for a description of inoculation procedures.

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 35 deg. C.

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. August, M.J., et al. 1990. *Cumitech 3A; Quality Control and Quality Assurance Practices in Clinical Microbiology*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Murray, P.R., et al. 1995. *Manual of Clinical Microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.

3. Forbes, B.A., et al. 2002. *Bailey and Scott's Diagnostic Microbiology*, 11th ed. C.V. Mosby Company, St. Louis, MO.

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

5. Koneman, E.W., et al. 1997. *Color Atlas and Textbook of Diagnostic Microbiology*, 5th ed. J.B. Lippincott Company, Philadelphia, PA.

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

072808md

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

Ordering Information

Distribution Centers:

California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida

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