IFU

Instructions for Use

BACTEROIDES BILE ESCULIN (BBE) AGAR

Cat. no. G05	BBE Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J102	BBE / LKV, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Bacteroides Bile Esculin Agar (BBE) is recommended for use in the selective isolation and presumptive identification of *Bacteroides fragilis* group.

SUMMARY

Livingston, et al., developed Bacteroides Bile Esculin Agar to accelerate the recognition of *Bacteroides fragilis* group by providing tentative identification from a primary plate medium within 48 hours.[8,11] The selective and differential media was developed by combining the components of twenty percent bile stimulation, esculin hydrolysis, catalase production, and kanamycin inhibition tests. Later, gentamicin was substituted for kanamycin. Gentamicin proved to be an effective substitute, as it does not lose its activity at incubation temperatures and can be incorporated into BBE Agar before autoclaving.[11]

The basal medium is composed of Tryptic Soy Agar (TSA) and is supplemented with the following: twenty percent bile (oxgall) to stimulate growth of *B. fragilis* group while inhibiting other anaerobes; esculin and ferric ammonium citrate to detect esculin hydrolysis; hemin which serves as a growth factor and allows testing for catalase production; and gentamicin which inhibits most facultative anaerobes.

B. fragilis group hydrolyzes esculin to form dextrose and esculetin. This compound reacts with the ferric ions contained within the medium, turning the medium around the colonies a dark brown to black color. Thus, the tolerance to the bile and hydrolysis of esculin provide the means to presumptively identify the *B. fragilis* group.

FORMULA

Ingredients per liter of deionized water:*

Tryptic Soy Agar	45.0gm
Oxbile (Oxgall)	20.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Hemin	12.0mg
Gentamicin	100.0mg

Final pH 7.0 +/- 0.3 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: To assure the recovery of anaerobes, specimens should be protected from air (oxygen) during collection, transport, and processing. Consult listed references for instructions concerning collection and transport of anaerobes.[1,2,4,6]

Method of Use: The medium should be warmed to room temperature and agar surface should be dry prior to inoculation. Inoculate and streak the medium to obtain growth of isolated colonies. Incubate in an anaerobic atmosphere at 35-37ºC. for 18-48 hours. Observe for growth and blackening of the medium.

INTERPRETATION OF RESULTS

This product is used in conjunction with other biochemical tests to identify cultures of isolated organism. Esculin hydrolysis is indicated by a browning or blackening in the medium surrounding a colony, which is indicative of the *Bacteroides fragilis* group.

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1,2.4,6]

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.

Some strains of *B. vulgatus*, a member of *B. fragilis* group, can test esculin-negative.

Esculin can be hydrolyzed by some bile-resistant, non-*B. fragilis* group microorganisms. Some examples of such microorganisms are *Odoribacter splanchnicus, Fusobacterium mortiferum, Klebsiella pneumoniae, Enterococcus* species, and yeasts. In general, *B. fragilis* group are two to three millimeters in size, whereas, the previously mentioned organisms are less than one millimeter in diameter.[9,10]

It is suggested that BBE Agar plates be reduced, prior to use, by placing them in an anaerobic atmosphere at room temperature for a period of 6-24 hours.

Many anaerobes are sensitive to oxygen during the log phase of growth and may be killed by exposure to oxygen before the colonies are fully developed. It may be necessary, therefore, to incubate an inoculated culture for 48 hours (three to five days is preferable) before exposing the culture to room air.

Some organisms which should grow on BBE Agar may be inhibited. It is recommended that a non-selective medium such as Brucella with H & K (Cat. no. A30) be inoculated in parallel in order to assure growth of all species present.

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, swabs, anaerobic generators and chambers, 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	A	18-24hr	35°C	Anaerobic	Growth; blackening of the media
Proteus mirabilis ATCC® 12453	B	18-24hr	35°C	Aerobic	Inhibited

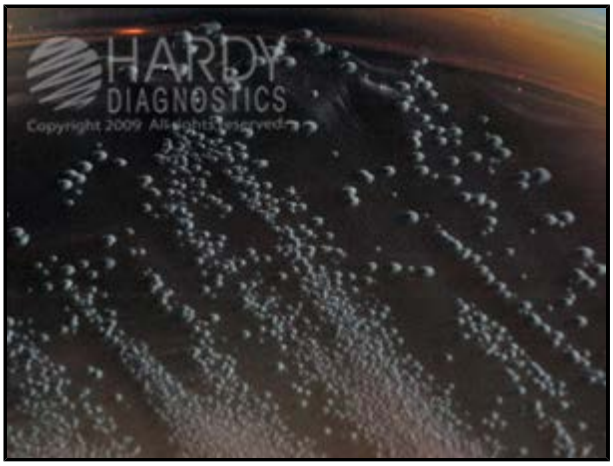
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

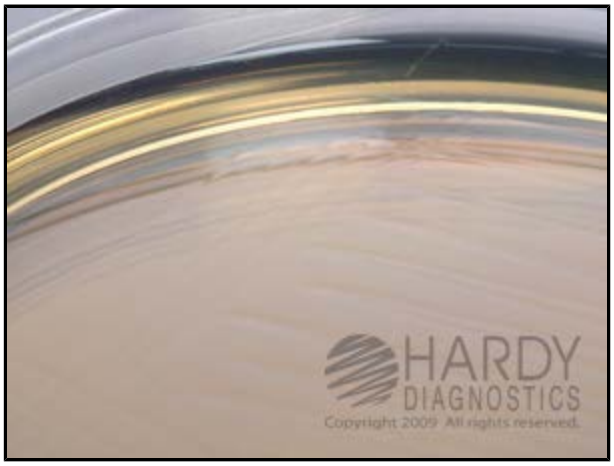
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

Bacteroides Bile Esculin (BBE) Agar should appear opalescent, and medium amber with blue tinge in color.



Bacteroides fragilis (ATCC® 25285) colonies growing on Bacteriodes Bile Esculin Agar (Cat. no. G05). Incubated anaerobically for 24 hours at 35ºC.



Proteus mirabilis (ATCC® 12453) growth inhibited on Bacteriodes Bile Esculin Agar (Cat. no. G05). Incubated aerobically for 24 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. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Facklam, R.R. and M.D. Moody. 1970. *Appl. Microbiol.*; 20:245

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

5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

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

7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22-A2. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS), Villanova, PA.

8. Dowell, V.R., Jr., et al. 1974. Laboratory Methods in Anaerobic Bacteriology, *CDC Lab. Manual*, USDHEW, Washington, D.C.

9. Finegold, S.M. and E.J. Baron. 1986. *Bailey and Scott's Diagnostic Microbiology*, 7th ed. C.V. Mosby, St. Louis, MO.

10. Lennette, E.H., et al. 1985. *Manual of Clinical Microbiology*, 4th ed. American Society for Microbiology Washington, D.C.

11. Livingston, S.J., et al. 1978. *J. Clin. Microbiol.*; 7:448-453.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10048[C]

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.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207F [D]