IFU

Instructions for Use

BILE ESCULIN AGAR (BEA)

Cat. no. G12	Bile Esculin Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. L10	Bile Esculin Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Bile Esculin Agar (BEA) is recommended for use as a differential medium in the isolation and presumptive identification of enterococci/group D streptococci.

SUMMARY

Esculin hydrolysis was first described by Rochaix in 1924.[8] Swan first introduced the use of Bile Esculin Agar in 1954.[9] In 1970, Facklam and Moody determined that the use of the bile esculin test was a reliable way of identifying group D streptococci from non-group D streptococci.[3] When using BEA in biochemical testing of group D streptococci, they found that all group D streptococci will blacken this medium.[3] Other researchers have used BEA for the presumptive identification of *Enterobacter* spp., *Klebsiella* spp., and *Serratia* spp., among the Enterobacteriaceae.

This medium contains esculin, ferric citrate to provide ferric ions, and 4% oxbile to inhibit most other strains of non-group D streptococci. Esculin is hydrolyzed by group D streptococci to form dextrose and esculetin. This compound reacts with the ferric ions contained within the medium, turning the medium from its original amber color to a dark brown to black. Thus the tolerance to the presence of bile and the hydrolysis of esculin provide the means to presumptively identify group D streptococci.

FORMULA

Ingredients per liter of deionized water:*

Oxbile (Oxgall)	40.0gm
Pancreatic Digest of Gelatin	5.0gm
Beef Extract	3.0gm
Esculin	1.0gm
Ferric Citrate	0.5gm
Agar	15.0gm

Final pH 6.6 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store plated media (Cat. no. G12) at 2-8ºC. away from direct light. Store tubed media (Cat. no. L10) at 2-30ºC. 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 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 organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Method of Use: Allow the BEA medium warm to room temperature before use. Inoculate and streak the medium with one isolated pure colony. Incubate in an aerobic atmosphere at 35ºC for 24-48 hours. Observe for growth and blackening of the medium.

INTERPRETATION OF RESULTS

Esculin hydrolysis is indicated by a blackening of the media around the colonies. 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 *Staphylococcus*, *Aerococcus*, and *Listeria monocytogenes* may grow in the presence of bile and hydrolyze esculin. *L. monocytogenes* will form minute black colonies.

A heavy inoculum on BEA may cause interpretation of the bile esculin test difficult to read. Excess inoculum decreases the ability of the bile to inhibit growth of other gram-positive organisms that may hydrolyze esculin.

There are a few streptococci that do not hydrolyze esculin but will grow in the presence of bile. Growth without blackening of this medium does not constitute a positive test.

BEA does not contain azide; as a result, gram-negative rods will grow on this medium. Many of these organisms may hydrolyze esculin.

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, microscope, incinerator, and incubators, 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	
*Enterococcus faecalis*** ATCC® 29212	A	24-48hr	35°C	Aerobic	Growth; blackening of media around colonies
*Streptococcus pyogenes*** ATCC® 19615	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

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

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

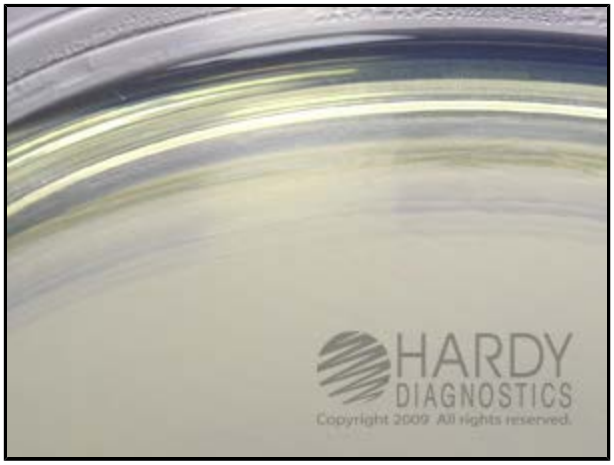
USER QUALITY CONTROL

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

Bile Esculin Agar should appear clear, and light amber with a bluish-tinge in color.

Enterococcus faecalis (ATCC® 29212) colonies growing on Bile Esculin Agar (Cat no. G12). Incubated aerobically for 24 hours at 35ºC.



Streptococcus pyogenes (ATCC® 19615) growth inhibited on Bile Esculin Agar (Cat no. G12). 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 & III. 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. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

8. Rochaix. 1924. *Cr. Soc. Biol.*, Paris; 90:771.

9. Swan, A. 1954. *J. Clin. Path.*; 7:160-163.

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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

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

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