IFU

Instructions for Use

BILE ESCULIN WITH AZIDE MEDIA

Cat. no. G11	Bile Esculin Agar (BEA) with Azide, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. K11	Bile Esculin Azide Broth, 13x100mm Tube, 5ml	20 tubes/box
Cat. no. J66	Bile Esculin Agar (BEA) with Azide / Columbia CNA, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Bile Esculin with Azide Media are recommended for the isolation and differentiation of group D streptococci from non-group D streptococci.

SUMMARY

Hardy Diagnostics Bile Esculin Agar with Azide is also referred to as Pfizer Selective Enterococcus (PSE) Agar. Esculin hydrolysis was first described by Rochaix in 1924.[8] Swan first introduced the use of Bile Esculin Agar in 1954.[9] Facklam and Moody, in 1970, 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*, *Klebsiella* and *Serratia* spp. only.

This medium contains esculin, ferric citrate to provide ferric ions, and 1% oxbile to inhibit most other strains of non-group D streptococci. Sodium azide is incorporated into the medium to inhibit gram-negative bacteria. 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 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:*

Pancreatic Digest of Casein	16.0gm
Oxbile	10.0gm
Yeast Enriched Meat Peptone	9.5gm
Sodium Chloride	5.0gm
Sodium Citrate	1.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm

Sodium Azide	0.25gm

In addition, Bile Esculin Agar (BEA) with Azide contains:

Agar	15.0gm

Final pH 7.1 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store Bile Esculin Agar with Azide at 2-8ºC. and Bile Esculin Azide Broth at 2-30ºC. Store media 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: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use: Allow the medium to warm to room temperature prior to inoculation. BEA with Azide is a primary isolation medium. This medium enhances the recovery and isolation of individual colonies from clinical specimens. Inoculate agar and streak for isolation using the four quadrant method. Using a sterile inoculating loop, inoculate broth with well isolated colonies. Incubate inoculated media in an aerobic atmosphere at 35ºC. for 18-48 hours. Observe for growth and blackening of the medium.

INTERPRETATION OF RESULTS

A positive test result denoting the presence of group D streptococci is any blackening of the media. A negative test result is no blackening of the media after 48 hours of incubation.

Consult listed references for information pertaining to colony morphology and 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* and *Aerococcus* can grow in the presence of bile and can hydrolyze esculin.

A heavy inoculum on Bile Esculin Azide Media may cause difficulties in interpreting the bile esculin test. Excess inoculum decreases the ability of the oxbile 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 for group D streptococci.

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, 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	
Bile Esculin Agar with Azide and Bile Esculin Azide Broth:					
Enterococcus faecalis ATCC® 29212	A	24-48hr	35°C	Aerobic	Growth; blackening of media around colonies or blackening of broth
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial inhibition; small colorless colonies. Growth inhibited in broth.
Streptococcus pyogenes ATCC® 19615	B	24-48hr	35°C	Aerobic	Partial to complete inhibition. Growth inhibited in broth.

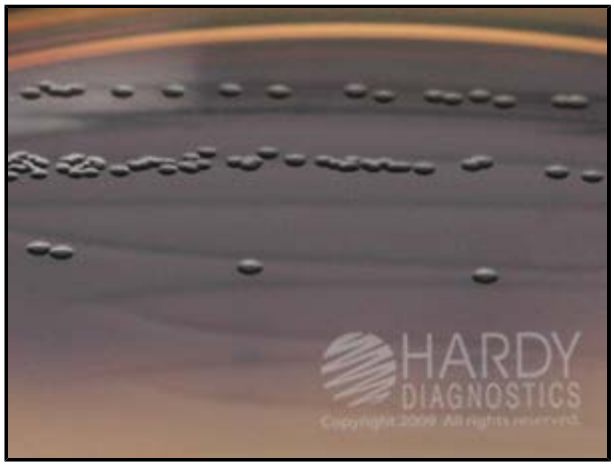
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

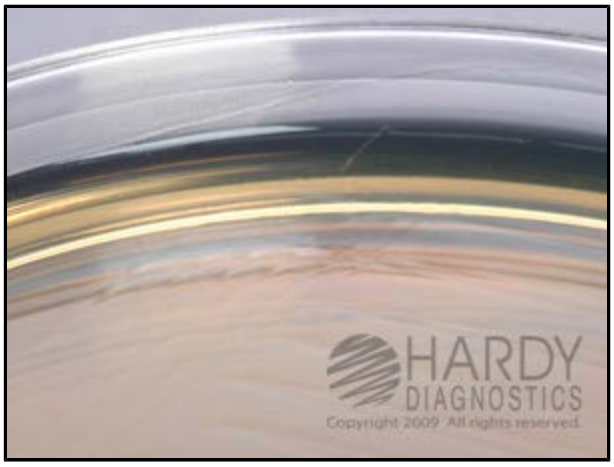
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory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

- Bile Esculin Agar (BEA) with Azide should appear clear, and light amber with a blue tinge in color.
- Bile Esculin Azide Broth should appear clear, and dark amber with a blue tinge in color.



Enterococcus faecalis (ATCC® 29212) colonies growing on Bile Esculin Agar with Azide (Cat. no. G11). Incubated aerobically for 24 hours at 35ºC.



Streptococcus pyogenes (ATCC®19615) growth inhibited on Bile Esculin Agar with Azide (Cat. no. G11). 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. McFaddin, 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, Cr. 1924. *Soc. Biol.*, Paris; 90:771.

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

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

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

HDQA 2207F [D]