IFU

Instructions for Use

BLOOD AGAR ENHANCED HEMOLYSIS (EH)

Cat. no. A03	Blood Agar EH, 5%, 15x100mm Plate, 17ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Blood Agar Enhanced Hemolysis (EH) is recommended for use as a general purpose growth medium for the isolation, cultivation, and differentiation of fastidious microorganisms from specimens where clear and distinct hemolytic reactions are of prime importance.

SUMMARY

Modified Tryptic Soy Agar is the basal medium for Blood Agar EH. This medium contains peptic digest of soybean meal, sodium chloride, agar, and modified pancreatic digest of casein (Tryptone H Plus). Sheep blood has been added to facilitate the growth of some organisms, and for the observation of hemolytic reactions. The absence of reducing sugars and carbohydrates allows hemolysis to occur without hindrance. Enhanced hemolysis is a result of Tryptone H Plus; this modified base enhances the production of hemolysin while minimizing antagonism or loss in activity of streptococcal hemolysins.[6]

Compared to traditional Blood Agar, Blood Agar EH produces larger, clearer zones of hemolysis groups A, C and G streptococci. Perrollaz et al. reported zone sizes to be 39% larger than with traditional media. Hemolysis was also found to develop more rapidly on Blood Agar EH.[7]

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Sheep Blood	50.0ml
Agar	12.0gm

Final pH 7.3 +/- 0.2 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), hemolysis, 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]

Prepared media should be inoculated, incubated, and results recorded according to accepted procedures described in the listed reference texts.[1-5]

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.

Since all hemolysis is enhanced, Blood Agar EH is not recommended for use with the CAMP test.

Zones of inhibition around Bacitracin Disks may be difficult to read.

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	
Streptococcus pneumoniae ATCC® 6305***	A	24hr	35°C	CO_2**	Growth, alpha-hemolysis
Streptococcus pyogenes ATCC® 19615***	A	24hr	35°C	CO_2**	Growth, beta-hemolysis
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	CO_2**	Growth, beta-hemolysis
Escherichia coli ATCC® 25922	A	24hr	35°C	CO_2**	Growth, beta-hemolysis

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

** Atmosphere of incubation is enriched with 5-10% CO_2.

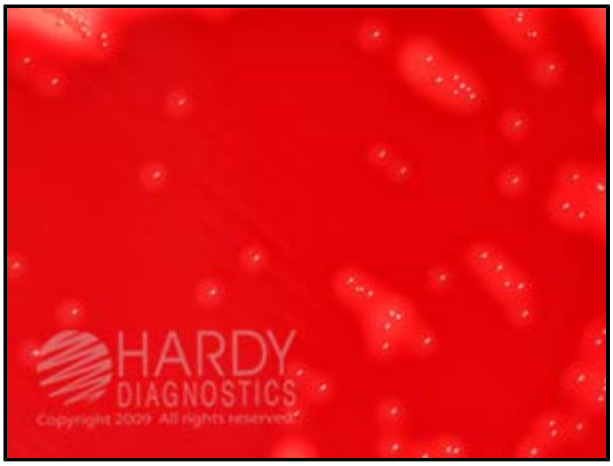
*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

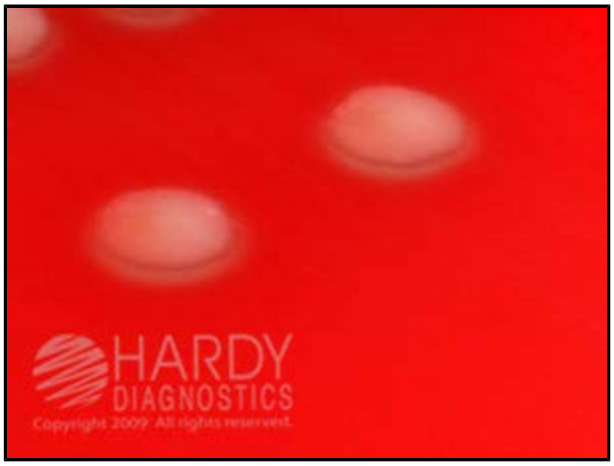
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory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

Blood Agar EH should appear opaque, and cherry red in color.

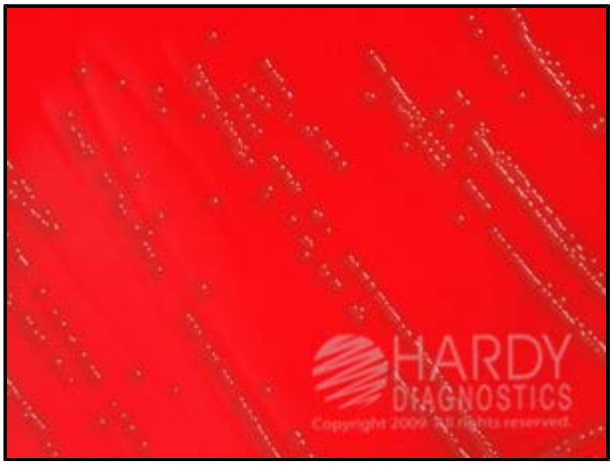


Streptococcus pyogenes (ATCC® 19615) colonies growing on Blood Agar EH, 5% (Cat. no. A03). Incubated in CO_2 for 24 hours at 35ºC.



Escherichia coli (ATCC® 25922) colonies growing on Blood Agar EH, 5% (Cat. no. A03). Incubated in CO_2 for 24 hours at 35ºC.

Staphylococcus aureus (ATCC® 25923) colonies growing on Blood Agar EH, 5% (Cat. no. A03). Incubated in CO_2 for 24 hours at 35ºC.



Streptococcus pneumoniae (ATCC® 6305) colonies growing on Blood Agar EH, 5% (Cat. no. A03). Incubated in CO_2 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. 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. *Bacto® Tryptic Soy Blood Agar Base EH*, Difco Laboratories, Detroit, MI, 1991.

7. Perrollaz, et al. 1992. *A Comparative Evaluation of Commercially Available Tryptic Soy and Columbia Blood Agar Base Media*, 1992 ASM General Meeting, New Orleans, LA.

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

HDQA 2207F [D]