IFU

Instructions for Use

HORSE BLOOD AGAR

Cat. no. A149	Horse Blood Agar 5%, 15x100mm Plate, 17ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Horse Blood Agar is an enriched all-purpose growth medium recommended for the cultivation of nonfastidious and fastidious microorganisms such as *Haemophilus* spp.

SUMMARY

Tryptic Soy Agar (TSA) is a popular all-purpose growth medium used to support a wide variety of microorganisms. TSA can be used unsupplemented or as a base for media containing mammalian blood. Although the most common form of blood containing media in the clinical laboratory is sheep blood agar, horse blood provides X- (hemin) and V-factors (NAD) required for the growth of fastidious microorganisms such as *Haemophilus influenzae*. Sheep and human blood are not suitable for this purpose, as they contain enzymes that inactivate NAD.[6]

Horse Blood Agar contains pancreatic digest of casein and soy peptone which provide essential carbon and nitrogen elements to support cell growth. Koenzyme enrichments are a chemically defined supplement that provides NAD (V-factor), amino acids, vitamins, dextrose, ferric ions and coenzymes to promote the growth of fastidious strains. Sodium chloride helps maintain osmotic equilibrium and agar is the solidifying agent. The medium is supplemented with 5% defibrinated horse blood to provide essential growth factors and to facilitate the determination of hemolytic reactions.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	14.5gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Koenzyme Enrichments	1.5ml
Horse Blood	50.0ml
Agar	14.0gm

Final pH 7.4 +/- 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, 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: 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 specific information on specimen collection and appropriate methods of culture.[2-6,8]

Method of Use:

1. Prior to inoculation, the medium should be brought to room temperature.

2. Inoculate the medium and streak the specimen to obtain isolated colonies.

3. Incubate at 35ºC. in a moist, CO_2 enriched atmosphere for 18-24 hours. NOTE: *H. aegyptius* requires a longer incubation period of 2-4 days. *H. ducreyi* may require up to 9 days incubation, preferably at 33ºC.

INTERPRETATION OF RESULTS

H. influenzae produce small, pale gray, moist non-hemolytic colonies with a characteristic "mousy" odor.

Haemophilus haemolyticus and *Haemophilus parahaemolyticus* may be similar in appearance to *H. influenzae* , except that colonies are surrounded by a zone of beta-hemolysis.

H. influenzae and *H. parainfluenzae* may be differentiated by colony color.[8]

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.

Defibrinated horse blood may give hemolytic reactions different from sheep blood.[5] Some streptococci (e.g. group D) give hemolytic reactions on horse blood, but not on sheep blood and may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organism should be tested with a Bacitracin differentiation disk (Cat. no. Z7021) and grouped serologically or tested by the fluorescent method. Beta-hemolytic streptococci and *Haemophilus haemolyticus* may be differentiated by performing a Gram stain from pure culture.

The medium is not recommended for use with throat cultures.[6]

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, swabs, applicator sticks, Bacitracin disk (Cat. no. Z7021), other culture media, 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	
Haemophilus influenzae ATCC® 10211***	B	18-24hr	35°C	CO_2**	Growth
Haemophilus influenzae ATCC® 49247	B	18-24hr	35°C	CO_2**	Growth
Streptococcus pyogenes ATCC® 19615***	B	18-24hr	35°C	CO_2**	Growth

* 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

Horse Blood Agar should appear opaque and cherry red in color.

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

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

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

6. Krumweide, E. and A.G. Kutter. 1938. A growth inhibitory substance for the influensa group of organisms in the blood of various animal species. The use of the blood of various animals as a selective medium for the detection of hemolytic streptococci in throat cultures. *J. Exp. Med*. 67:429-441.

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

8. Roberts, D.E., E. Higgs and P.J. Cole. 1987. Selective medium that distinguishes *Haemophilus influenzae* from *Haemophilus parainfluenzae* in clinical specimens: its value in investigating respiratory sepsis. *J. Clin. Pathol*. 40:75-76.

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

HDQA 2207F [D]