

Instructions for Use

CHOCOLATE AGAR

Cat. no. E14	Chocolate Agar, 15x100mm Plate, 19ml	10 plates/bag
Cat. no. E14BX	Chocolate Agar, 15x100mm Plate, 19ml	100 plates/box
Cat. no. H25	Chocolate Agar, 15x150mm Plate, 69ml	10 plates/bag
Cat. no. J42	Blood Agar, 5% / Chocolate Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J44	Chocolate Agar / Martin Lewis Agar with Lincomycin, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J72	Chocolate Agar / Modified Thayer Martin (MTM) Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. L37	Chocolate Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Chocolate Agar is recommended for use in the isolation and cultivation of fastidious microorganisms, particularly *Haemophilus* and *Neisseria* species.

SUMMARY

In the late 1920s, McLeod et al. developed Chocolate Agar using a formulation incorporating yeast extract and peptones.⁽⁹⁾ The development of this medium provided the ground work for the improvement of culture methods for fastidious microorganisms. In 1945, Johnston reported a medium which produced *Neisseria gonorrhoeae* colonies successfully within 24 hours.⁽⁶⁾ The recovery time of *N. gonorrhoeae* was further decreased by Carpenter and Morton in 1947 when they added GC Agar Base, enriched with hemoglobin and yeast extract, to the Chocolate Agar formulation.⁽⁴⁾ The accelerated growth of *N. gonorrhoeae* was attributed to differences in gel strength, thus the new formulation contained less agar than previously indicated.⁽⁶⁾ The medium was further improved in 1967 by Martin et al. by replacing the yeast concentrate with a chemically defined enrichment (KoEnzyme Enrichment) designed to aid the growth of *Neisseria* species.⁽⁸⁾

Chocolate Agar consists of GC Agar Base with added hemoglobin and KoEnzyme Enrichment. GC Agar Base contains proteose peptone which provides nitrogenous nutrients. Hemoglobin releases hemin (X-factor) components. The phosphate buffer is added to maintain the pH of the medium. Corn starch aids in neutralizing any toxic fatty acids present. KoEnzyme Enrichment is a chemically defined supplement that provides NAD (V-factor), amino acids, vitamins, dextrose, ferric ions, and coenzymes to promote the growth of *Neisseria* species.

FORMULA

Ingredients per liter of deionized water:*

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Chocolate Agar:	
Proteose Peptone	15.0gm
Sodium Chloride	5.0gm
Dipotassium Phosphate	4.0gm
Monopotassium Phosphate	1.0gm
Corn Starch	1.0gm
Hemoglobin, Bovine	10.0gm
KoEnzyme Enrichment	10.0ml
Agar	10.0gm
KoEnzyme Enrichment:	
Dextrose	100.0gm
L-Cysteine, HCl	25.9gm
L-Glutamine	10.1gm
L-Cystine	1.1gm
Adenine	1.0gm
Nicotinic Adenine Dinucleotide	0.25g
Coccarboxylase	0.1g
Guanine Hydrochloride	30.0gm
Ferric Nitrate	20.0mg
P-Aminobenzoic Acid	13.0mg
Vitamin B ₁₂	10.mg
Thiamine	3.0mg

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. 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: Consult listed references for information on specimen collection.^(1-3,5,7) 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 maintained at an appropriate temperature.^(1-3,5,7)

Method of Use: Inoculate the Chocolate Agar and streak the specimen as soon as possible after it is received in the clinical laboratory. If the specimen is being cultured directly from a swab, roll the swab over a small portion of the agar surface, and streak for isolation. Incubate in 5-10% CO₂ at 35-37°C. for 24 to 48 hours. Extend incubation if needed. Subcultures of *N. gonorrhoeae* should be made within 18-24 hours.

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of fastidious species.^(1-3,5,7)

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.

Chocolate Agar is an enriched medium, thus non-pathogenic organisms may overgrow pathogenic bacteria. If isolation of *N. gonorrhoeae* is desired, a selective medium such as Thayer Martin Agar, Modified (Cat. no. E30), or Martin Lewis Agar with Lincomycin (Cat. no. E39) should be used in parallel with this non-selective formula.

Precipitated hemoglobin may appear as dark spots on or in the media and does not affect the performance of the media.

The presence or absence of *N. gonorrhoeae* in a specimen does not rule out the possible presence of other pathogenic organisms.

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	
<i>Neisseria gonorrhoeae</i> ATCC® 43069	A	24hr	35°C	CO ₂ **	Growth
<i>Haemophilus influenzae</i> ATCC® 10211	A	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% CO₂.

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

Chocolate Agar should appear opaque, and brown in color.



Haemophilus influenzae (ATCC® 10211) colonies growing on Chocolate Agar (Cat. no. E14) Incubated in CO₂ for 24 hours at 35°C.



Neisseria gonorrhoeae (ATCC® 43069) colonies growing on Chocolate Agar (Cat. no. E14) Incubated in CO₂ for 24 hours at 35°C.



Uninoculated plate of Chocolate Agar (Cat. no. E14).

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. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
3. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Carpenter, C.M. and H.E. Morton. 1947. *Proc. N.Y. State Assoc. Public Health Labs*; 27:58-60.
5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
6. Johnston, et al. 1945. *J. Ven. Dis. Inf.*; 26:239.
7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*. J.B. Lippincott Company, Philadelphia, PA.
8. Martin, J.E., et al. 1967. *Public Health Rep.*; 82:361.
9. McLeod, J.W., et al. 1927. *Br. J. Exp. Pathol.*; 8:25.
10. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

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