

Instructions for Use

COLUMBIA BLOOD AGAR

Cat. no. A16	Columbia Blood Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. A16BX	Columbia Blood Agar, 15x100mm Plate, 18ml	100 plates/box

INTENDED USE

Hardy Diagnostics Columbia Blood Agar is recommended for use as a general growth medium for the cultivation and hemolytic determination of fastidious and non-fastidious microorganisms from clinical specimens.

SUMMARY

Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford, and Vasi who incorporated animal derived peptone, enzymatic digests of casein, and defibrinated sheep blood into one medium.⁽³⁾ It was found to be an improved blood agar, promoting both luxuriant and rapid growth, improved pigment production, typical colony morphology, and sharply defined hemolytic reactions.

REAGENT FORMULA

Ingredients per liter of deionized water:*

Enzymatic Digests of Casein	10.0gm
Casein Yeast Peptone	10.0gm
Sodium Chloride	5.0gm
Tryptic Digest of Beef Heart	3.0gm
Corn Starch	1.0gm
Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.3 +/- 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.

Specimen Collection: Consult listed references for information on specimen collection.^(1,2,4,6) 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.

Method of Use: Allow the plates to warm to room temperature, and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically or in a 5% to 10% carbon dioxide atmosphere at 35-37°C. for 18-24 hours. Examine colonial morphology, characteristics, and hemolytic reactions.

INTERPRETATION OF RESULTS

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 beta-hemolytic streptococci strains will develop green hemolytic zones on Columbia Blood Agar. It is recommended to subculture all such streptococci strains onto Blood Agar plates (Cat. no. A10) to verify this reaction.

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>Streptococcus pyogenes</i> ATCC® 19615	A	24hr	35°C	Aerobic	Growth, beta-hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	A	24hr	35°C	Aerobic	Growth, alpha-hemolysis
<i>Staphylococcus aureus</i> ATCC® 25923	A	24hr	35°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth

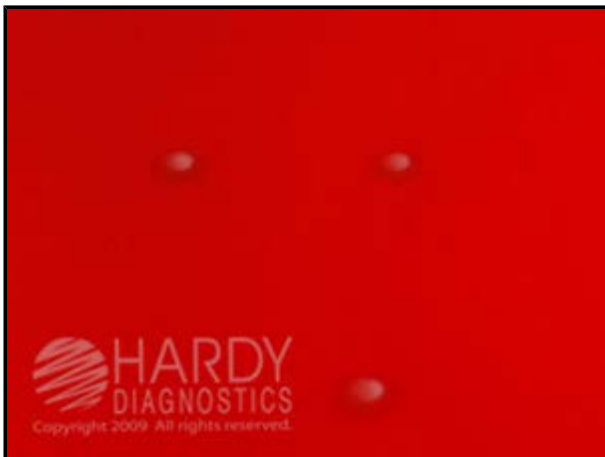
* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

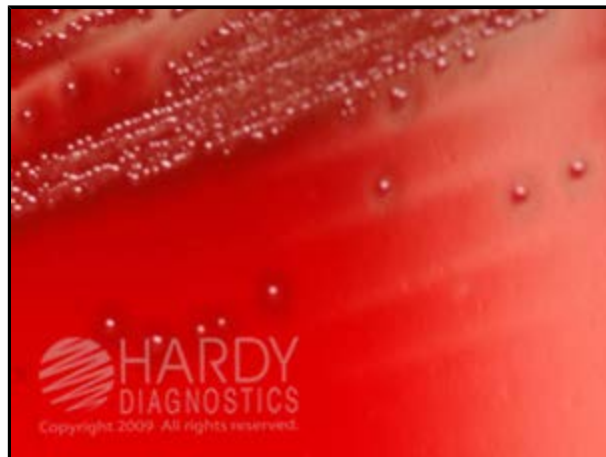
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory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

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



Streptococcus pyogenes (ATCC® 19615) colonies growing on Columbia Blood Agar (Cat. no. A16). Incubated aerobically for 24



Streptococcus pneumoniae (ATCC® 6305) colonies growing on Columbia Blood Agar (Cat. no. A16). Incubated aerobically for 24

hours at 35°C.



Staphylococcus aureus (ATCC® 25923) colonies growing on Columbia Blood Agar (Cat. no. A16). Incubated aerobically for 24 hours at 35°C.

hours at 35°C.



Escherichia coli (ATCC® 25922) colonies growing on Columbia Blood Agar (Cat. no. A16). Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of Columbia Blood Agar (Cat. no. A16).

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.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. Ellner, et al. 1966. *Am. Journ. Clin. Path.*; 45:502.
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. Versalovic, J., 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.

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

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