IFU

Instructions for Use

CRITERION™ CLED AGAR

Cat. no. C5400	CRITERION™ CLED Agar	72gm
Cat. no. C5401	CRITERION™ CLED Agar	500gm
Cat. no. C5402	CRITERION™ CLED Agar	2kg
Cat. no. C5403	CRITERION™ CLED Agar	10kg
Cat. no. C5404	CRITERION™ CLED Agar	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ CLED Agar is recommended for the isolation and enumeration of microorganisms from urine.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

In the mid 1960s, Sandys and Mackey reported on the laboratory diagnosis of urinary tract infections using a new medium Sandys had developed to prevent the swarming of *Proteus* spp.[7,8,10] Previous culture methods used to inhibit the swarming of *Proteus* included adding chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid, and sulfonamides to the medium.[10] However, Mackey and Sandys' modified medium replaced mannitol with lactose, discontinued the use of sucrose, increased the indicator strength of bromothymol blue and the concentration of agar, and incorporated the use of cystine in order to enhance the growth of cystine-dependent "dwarf colony" coliforms.[8] They named their final medium Cystine Lactose Electrolyte-Deficient (CLED) Agar and reported it as ideal for dip-inoculum techniques and for general urinary bacteriology and colony differentiation. CLED Agar also lacks sodium chloride, which helps in preventing the swarming of *Proteus* spp.

CLED Agar supports the growth of all potential urinary pathogens, and a number of contaminants such as diphtheroids, lactobacilli, and micrococci. Urine samples containing mixed flora are typical of urethral or vaginal contamination.

Research demonstrates that the best results are obtained when inoculation occurs as soon after sample collection as possible.[2,8] Otherwise, confluent or semiconfluent growth may occur when CFU counts exceed 10^5 per ml of urine. Historically, reliable quantitative urine cultures have been obtained using the dip-inoculum method, even when there was a time delay of several hours between inoculation of urine onto the medium and incubation.[2] Therefore, the inoculated medium may be held for 48 hours or longer, refrigerated or at room temperature, until received by a testing facility. This makes CLED Agar extremely versatile for physicians' offices, small clinical labs, or hospital wards, and eliminates the need for transport and refrigeration of patient urine specimens.

Hardy Diagnostics CRITERION™ CLED Agar is recommended for use in the spread plate technique or the dip-inoculum method for detection of bacteria in urine. CRITERION™ CLED Agar contains enzymatic digest of casein,

enzymatic digest of gelatin, and beef extract, which provide nitrogen, vitamins, and carbon to support microbial growth. Lactose is added as the carbohydrate source. L-cystine is a growth supplement for cystine-dependent coliforms. Organisms capable of fermenting lactose will lower the pH and change the color of the medium to yellow. Consequently, bromothymol blue is the pH indicator. Agar acts as the solidifying agent.

FORMULA*

Gram weight per liter:	36.0gm/L
Lactose	10.0gm
Pancreatic Digest of Gelatin	4.0gm
Pancreatic Digest of Casein	4.0gm
Beef Extract	3.0gm
L-Cystine	0.128gm
Bromothymol Blue	0.02gm
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light bluish-tan.

Store the prepared culture media at 2-8ºC.

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 36gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat to boiling for one minute to dissolve completely.

3. Check the pH and adjust if necessary.

4. Sterilize in the autoclave at 121ºC. for 15 minutes.

5. Cool to 45-50ºC.

6. Aseptically pour into desired sterile containers.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed reference or refer to the prepared media Instructions for Use (IFU) for Cat. No. G223.

LIMITATIONS

Limiting factors in low urine counts from infected patients include: rapid rate of urine flow, prior initiation of antimicrobial treatment, urine with a pH less than 5 and a specific gravity less than 1.003.[8]

The nutritional requirements of organisms vary and some strains may grow poorly or fail to grow entirely on this medium.

CRITERION™ CLED Agar is a non-selective medium. However, the growth of some *Shigella* species may be inhibited due to electrolyte exclusion in the formula.[6]

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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	Incubation			Results

	Method*	Time	Temperature	Atmosphere	
Enterococcus faecalis ATCC® 29212	A	24hr	35°C	Aerobic	Growth; small yellow colonies
Escherichia coli ATCC® 25922	A	24hr	35°C	Aerobic	Growth; yellow colonies, opaque, center slighlty deeper yellow
Proteus mirabilis ATCC® 12453	A	24hr	35°C	Aerobic	Growth; translucent blue colonies
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	Aerobic	Growth; deep yellow colonies
Pseudomonas aeruginosa ATCC® 27853	A	24hr	35°C	Aerobic	Growth; green colonies with matte surface and rough periphery
Klebsiella pneumoniae ATCC® 13883	A	24hr	35°C	Aerobic	Growth; yellow to whitish-blue colonies, mucoid

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

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ CLED Agar powder should appear homogeneous, free-flowing, and light bluish-tan in color. The prepared media should appear slightly opalescent, and light blue-green 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. Benner, E.J. 1970. Simple Disposable Method for Quantitative Cultures of Urine. *Appl. Micro.* Vol. 19, No. 3.

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. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. Mackey, J.P and G.H. Sandys. 1965. Laboratory Diagnosis of Infections of the Urinary Tract in General Practice by Means of a Dip-inoculum Transport Medium. *Brit. Med. J.*; 2:1286-1288.

8. Mackey, J.P. and G.H. Sandys. 1966. Diagnosis of Urinary Infections. *Brit. Med. J.*; 1:1173.

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

10. Sandys, G.H. 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routing laboratory practice. *J. Med. Lab. Technol.*; 17:224.

IFU-10132[A]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207B [D]