IFU

Instructions for Use

CLED AGAR

Cat. no. G223	CLED Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J315	Blood Agar / CLED Agar / MacConkey Agar, 15x100mm Triplate, 7ml/section	10 plates/bag
Cat. no. J65	Blood Agar 5% / CLED w/Bile Agar, 15x100mm Biplate, 10ml/section	10 plates/bag

INTENDED USE

Hardy Diagnostics CLED Agar is recommended for the isolation, enumeration, and presumptive identification of urinary pathogens on the basis of lactose fermentation, while controlling the swarming of *Proteus* spp.

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-slide techniques and for general urinary bacteriology and colony differentiation. CLED Agar also lacks sodium chloride, which helps to prevent 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 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.

Hardy Diagnostics CLED Agar is recommended for use in the spread plate technique for detection of bacteria in urine. The medium 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. The addition of bile salts in CLED Agar with Bile further suppresses growth of Gram-positive bacteria, including *Staphylococci*, which may be present in the specimen.

FORMULA

Ingredients per liter of deionized water:*

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.

In addition: CLED Agar with Bile contains 1.5g of bile salts.

* 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), 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

It is recommended that quantitative methods be used for cultivating specimens obtained from urine. For best results, the medium should be inoculated with a 1ul loop of a freshly voided, "clean catch" mid-stream urine as soon as possible after receipt. If there is a delay in getting the specimen to the lab, it should be refrigerated until inoculation to the media is possible. Incubate the media at 35 +/- 2ºC. for 24-48 hours. Consult the appropriate references for additional information on inoculation and incubation methods as established by laboratory policy.[1,3,4,9]

If only a small volume of urine is available, a small sample (approximately 1ml) may be poured directly over the medium, and the plate swirled to coat the entire surface for inoculation.

INTERPRETATION OF RESULTS

Count the number of colonies on the plate. Multiply the result by the appropriate number to convert the count to the number of CFU per ml of urine sample.

Potential bacterial contaminants usually appear in low numbers and vary in morphology. Typical urinary pathogens will usually yield high counts, have uniform colony morphologies, and should be subcultured directly to routine media for further identification and susceptibility testing.

Typical colony morphology on CLED Agar is as follows:

Escherichia coli	Opaque yellow colonies with a slightly deeper yellow center
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid[7]
Proteus	Translucent blue colonies
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery
Enterococci	Small yellow colonies, about 0.5mm in diameter
Staphylococcus aureus	Deep yellow colonies, uniform in color
Staphylococci coagulase-negative	Pale yellow colonies, more opaque than *Enterococcus faecalis*

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.

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.

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]

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, 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	Incubation			Results

	Method*	Time	Temperature	Atmosphere	
CLED Agar (Cat. no. G223 and J315):					
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
CLED Agar with Bile (Cat. no. J65):					
Escherichia coli ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth; yellow colonies with yellow halo
Enterococcus faecalis ATCC® 29212	B	18-24hr	35°C	Aerobic	Inhibition
Klebsiella pneumoniae ATCC® 13883	A	24hr	35°C	Aerobic	Yellow to whitish-blue colonies, mucoid

* 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

CLED Agar should appear clear, slightly opalsescent, light blue-green in color.
CLED Agar w/Bile should appear clear, slightly opalescent, light blue-green in color with or without slight precipitate.

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 Medical 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.

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

HDQA 2207F [D]