MEDIA FOR DETECTING FLUOROQUINOLONE NON-SUSCEPTIBLE MICROORGANISMS

Cat. no. G258	MacConkey Agar with Ciprofloxacin, 10µg/ml, 15x100mm Plate, 19ml	10 plates/bag
Cat. no. K258	BHI Broth with Ciprofloxacin, 10µg/ml, 13x100mm Glass Tube, 5ml	20 tubes/box

INTENDED USE

Hardy Diagnostics BHI Broth with Ciprofloxacin, 10µg/ml is a selective pre-enrichment broth for gram-negative microorganisms with reduced susceptibility to ciprofloxacin. Pre-enrichment with the broth is performed with a mixed population of microorganisms from a lab sample. Hardy Diagnostics MacConkey Agar with Ciprofloxacin, 10µg/ml is a companion product meant to act as a screening subculture medium for the selective isolation of non-susceptible Ciprofloxacin, gram-negative microorganisms. Concomitant cultures are necessary to recover microorganisms for identification, susceptibility testing, and epidemiological typing. A negative result does not preclude fluoroquinolone non-susceptible, gram-negative colonization. Culture results obtained from these products are not intended to guide therapy.

SUMMARY

Microbial antibiotic resistance has increased significantly among gram-negative bacilli in recent years and colonization of the gastrointestinal tract by resistant microorganisms serves as a reservoir for person-to-person transmission, as well as a likely source of potential clinical infection. The latter source is of particular importance for patients undergoing transrectal prostate biopsy in the histological diagnosis of prostate carcinoma. Approximately 800,000 biopsies are performed in the U.S. each year and complications from this procedure include urinary tract infections, prostatitis, and sepsis.[2,7-9] In general, the procedure usually follows antibiotic prophylaxes with fluoroquinolones and patients harboring fluoroquinolone-resistant microorganisms are at greater risk of contracting post-procedural infection.

The most common organism responsible for bacterial infection and clinical complication following transrectal prostate biopsy is *Escherichia coli*. Consequently, selective broth pre-enrichment, followed by selective culture on solid agar, is a useful tool in identifying patients colonized with resistant microorganisms prior to biopsy.[2,7-9]

Hardy Diagnostics media for detecting fluoroquinolone non-susceptible microorganisms are meant to be used for the selective pre-enrichment and isolation of fluoroquinolone non-susceptible enteric bacteria. Liquid media containing ciprofloxacin is meant to be used as a selective pre-enrichment broth from a mixed sample before subculture of the sample to solid media containing the same concentration of antibiotic. Ciprofloxacin is added to the media at 10µg/ml to aid in the selective isolation of fluoroquinolone non-susceptible microorganisms. BHI Broth with Ciprofloxacin, 10µg/ml (Cat.no. K258) should only be used as a selective pre-enrichment step with MacConkey Agar with Ciprofloxacin, 10µg/ml (Cat. no. G258) for best results.

FORMULA

Ingredients per liter of deionized water:*

MacConkey Agar with Ciprofloxacin, 10µg/ml (Cat. no. G258):	
Peptone	17.0gm
Lactose	10.0gm
Sodium Chloride	5.0gm
Proteose Peptone	3.0gm
Bile Salts	1.5gm
Neutral Red	30.0mg
Ciprofloxacin**	10.0mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.1 +/- 0.2 at 25ºC.

BHI Broth with Ciprofloxacin, 10µg/ml (Cat. no. K258):	
Calf Brain-Beef Heart Infusion	17.5gm
Pancreatic Digestion of Gelatin	10.0gm
Sodium Chloride	5.0gm
Disodium Phosphate	2.5gm
Dextrose	2.0gm
Ciprofloxacin***	0.01gm

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 for agar media) 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 dating 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 quality control incubation times.

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 *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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection and culture.[3-10] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

Method of Use:

1. Collect rectal swab specimen and place the swab directly into a BHI Broth with Ciprofloxacin, 10µg/ml (Cat. no. K258) tube.* If swabs are collected in the examination room, they should be placed into the BHI Broth with Ciprofloxacin tube and transported at room temperature to the laboratory without delay.

2. Incubate the swab in BHI Broth with Ciprofloxacin with slightly loose caps at 35 +/- 2ºC. for 24 hours.

3. Transfer 0.1ml of the BHI Broth with Ciprofloxacin culture to a MacConkey Agar with Ciprofloxacin plate (Cat. no. G258) and streak the specimen using a sterile loop to obtain isolated colonies.

4. Incubate plates in ambient air at 35 +/- 2ºC. for 24 to 48 hours and examine plates for colony growth. Colonies growing as gram-negative, enteric bacilli suspected of being fluoroquinolone non-susceptible *Escherichia coli* should be submitted for further biochemical confirmatory and susceptibility testing.

*Alternatively, direct plating of rectal swabs to MacConkey Agar with Ciprofloxacin has been described in the literature.[12]

INTERPRETATION OF RESULTS

Positive growth of BHI Broth with Ciprofloxacin cultures subbed to MacConkey Agar with Ciprofloxacin plates showing the growth of gram-negative enteric bacilli shall be suspected of presenting reduced susceptibility to fluoroquinolone antibiotics. Concomitant cultures are necessary to recover organisms for identification, susceptibility testing, and epidemiological typing. A negative result does not preclude fluoroquinolone non-susceptible, gram-negative colonization. Culture results obtained from these products are not intended to guide therapy.

LIMITATIONS

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

MacConkey Agar with Ciprofloxacin is recommended for selective isolation of gram-negative bacilli with reduced susceptibility to fluoroquinolone antibiotics. The formulation is not intended for use as a method of antimicrobial susceptibility testing or to guide therapy.

Confirmation of resistance by an approved method is necessary, as some organisms on initial isolation may overcome the inhibitory effecs of this medium.

The absence of suspect colonies on MacConkey Agar with Ciprofloxacin medium does not rule out the presence of fluoroquinolone non-susceptible microorganisms.

If no growth of positive BHI Broth with Ciprofloxacin cultures subbed to MacConkey Agar with Ciprofloxacin plates is observed after 24 hours, allow plates to incubate for up to 48 hours before determining negative cultures.[6]

Greater sensitivity of low-level fluoroquinolone-resistance has been shown by direct plating of stool and perirectal cultures to MacConkey Agar supplemented with greater than or equal to 0.125µg/ml levofloxacin and incubated in 5 to

10% CO_2; consequently, culture of perirectal or rectal swabs alone may fail to identify fluoroquinolone-resistant organisms in patients with low-level colonization.[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, pipets, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
MacConkey Agar with Ciprofloxacin, 10µg/ml (Cat. no. G258):					
Acinetobacter baumannii ATCC® BAA-1605**	A	24hr	35°C	Aerobic	Growth; colorless to slightly pink colonies
Escherichia coli ATCC® 25922**	B	24hr	35°C	Aerobic	Inhibited
Enterococcus faecalis ATCC® 29212	B	24hr	35°C	Aerobic	Partial to complete inhibition
BHI Broth with Ciprofloxacin, 10µg/ml (Cat. no. K258):					
Acinetobacter baumannii ATCC® BAA-1605**	A	24hr	35°C	Aerobic	Growth; broth becomes turbid
Escherichia coli ATCC® 25922**	B	24hr	35°C	Aerobic	No growth; broth remains the same

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

** 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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

MacConkey Agar with Ciprofloxacin, 10µg/ml should appear clear, slightly opalescent, and reddish-purple in color. BHI Broth with Ciprofloxacin, 10µg/ml should appear clear, and medium amber 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. Batura, D., G.G. Rao, and P.B. Nielsen. 2010. Prevalence of Antimicrobial Resistance in Intestinal Flora of Patients Undergoing Prostatic Biopsy; Implications for Prophylaxis and Treatment of Infections After Biopsy. *BJU Int.*; 106:1017-1020.

3. Tille, P.M., 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. Lautenbach, E., A.D. Harris, E.N. Perencevich, I. Nachamkin, P. Tolomeo, and J.P. Metlay. 2005. Test Characteristics of Perirectal and Rectal Swab Compared to Stool Sample for Detection of Fluoroquinolone-Resistant *Escherichia coli* in the Gastrointestinal Tract. *Antimicro. Agents and Chemo.*; 49(2):798-800.

7. Liss, M.A., A.N. Peeples, L. Pham, and E.M. Peterson. 2010. Detection of Fluoroquinolone Resistant Organisms from Rectal Swabs. Presented at the 2010 American Society for Microbiology General Meeting. San Diego, CA.

8. Liss, M.A., A.N. Peeples, and E.M. Peterson. 2011. Detection of Fluoroquinolone-Resistant Organisms from Rectal Swabs by Use of Selective Media Prior to a Transrectal Prostate Biopsy. *J. Clin. Micro.*; 49(3):1116-1118.

9. Liss, M.A., A. Chang, R. Santos, A.N. Peeples, E.M. Peterson, K. Osann, J. Billimek, R.J. Szabo, and A. Dash. 2011. Prevalence and Significance of Fluoroquinolone Resistant *Escherichia coli* in Patients Undergoing Transrectal Ultrasound Guided Prostate Needle Biopsy. *J. Urol.*; 185(4):1283-1288.

10. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

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

12. Liss, M.A., K.K. Nakamura, and E.M. Peterson. 2013. Comparison of Broth Enrichment to Direct Plating for Screening of Rectal Cultures for Ciprofloxacin-Resistant *Escherichia coli*. *J. Clin. Microbiol*. 51(1):249.

13. Bryan, L.G., K.C. Carroll, J. Miller, A. Thompson. 2013:D-1146 ICAAC Poster. Evaluation of Selective Media to Survey for Ciprofloxacin-Resistant Gram negative Bacterial Colonization in Patients Undergoing Prostate Biopsy. www.icaaconline.com accessed 26-May-2015.

IFU-10562[B]

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