IFU

Instructions for Use

EC BROTH WITH DURHAM TUBE

Cat. no. K13	EC Broth with Durham Tube, 20x125mm Tube, 13ml	20 tubes/box
Cat. no. K63	EC Broth with Durham Tube, 16x125mm Tube, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics EC Broth with Durham Tube is recommended for the detection of fecal coliforms at 44.5 and 45.5ºC.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Hajna and Perry formulated EC Broth for use in the examination of waters, milk, shellfish and other material for evidence of fecal pollution.[12] The medium consists of a buffered lactose broth with casein peptones and bile salts.

Lactose in the medium serves as a source of fermentable carbohydrate for the growth of coliforms. Casein peptones provide a source of nutrients. The bile salts serve as inhibitory agents toward gram-positive cocci and spore formers, particularly fecal streptococci and bacilli. The pH of the medium is maintained by the presence of a strong potassium buffering system.

Lactose-fermenting microorganisms that are capable of growing in the presence of the bile salts will ferment lactose which in turn results in the production of gas. Gas production is noted by the appearance of bubbles within the durham tube. Incubation at elevated temperatures is used to distinguish organisms of the fecal coliform group from total coliforms. For the testing of water, wastewater and shellfish, the development of turbidity and gas production within 24 hours at 44.5ºC indicates the presence of fecal coliforms.[1] When testing food other than shellfish, the development of turbidity and gas production within 48 hours at 45.5ºC indicates the presence of fecal coliforms.[4]
All shellfish isolates require an incubation temperature of 44.5ºC (rather than 45.5).[4]

The American Public Health Association (APHA) recommends that EC Broth be used in the fecal coliform. Most Probable Number (MPN) procedure for the examination of water, wastewater and foods.[1-3]

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	20.0gm
Lactose	5.0gm
Sodium Chloride	5.0gm
Dipotassium Phosphate	4.0gm
Monopotassium Phosphate	1.5gm
Bile Salts No. 3	1.5gm

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store the product at 2-30ºC. Products should not be used if there are any signs of contamination, deterioration, 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 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.

PROCEDURE

Sample Collection: Consult listed references for information on sample collection.[1-5]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning inoculation procedures.[1-5]

INTERPRETATION OF RESULTS

Gas production with growth within 24 +/- 2 hours of incubation at 44.5 +/- 0.2ºC is considered positive for the presence of fecal coliforms in water and wastewater. Absence of gas production is considered a negative test for the presence of fecal coliforms.

Production of gas within 48 +/- 2 hours of incubation at 45.5 +/- 0.2ºC is considered positive evidence of fecal coliforms in foods other than shellfish. Absence of gas production is considered a negative test for the presence of fecal coliforms.

Consult listed references for detailed results for the confirmation of *E. coli* and enumeration of coliforms using EC Broth.[2,4] For water and wastewater testing, a positive result is considered a confirmed result and no further testing is required.[1]

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.

It may be required that further testing be performed for confirmed results.

EC Broth should not be used for the direct isolation of coliforms.

Prior enrichment in a presumptive medium is required for optimal recovery of fecal coliforms when using EC Broth.

It may be necessary to invert the tube prior to inoculation if bubbles are trapped in the durham tube. Trapped bubbles that are not released may lead to false-positive results.

Turbidity alone is not indicative of a positive test for the presence of coliforms; turbidity with gas production is considered a positive test.

Incubation at 44.5 +/- 0.2ºC is recommended for the detection of fecal coliforms in water and wastewater.

Incubation at 45.5 +/- 0.2ºC is recommended for the detection of fecal coliforms in foods other than shellfish.

All shellfish isolates require an incubation temperature of 44.5ºC (rather than 45.5).[4]

As growth requirements for organisms vary, some strains may be encountered which fail to grow, or grow poorly on this medium.[6,7]

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	
Escherichia coli ATCC® 25922**	A	24hr	35°C	Aerobic	Growth; turbidity with gas production (bubble in durham tube)
Enterococcus faecalis ATCC® 29212**	B	24hr	35°C	Aerobic	Inhibited

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

** Recommended QC strains.

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

EC Broth with Durham Tube should appear clear, and colorless to light amber in color.

Escherichia coli (ATCC® 25922) growing in EC Broth with Durham Tube (Cat. no. K13). The bubble in the Durham tube indicates gas production. Incubated aerobically for 24 hours at 35ºC.

Enterococcus faecalis (ATCC® 29212) inhibited in EC Broth with Durham Tube (Cat. no. K13). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

3. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, American Public Health Association, Washington, D.C.

4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

5. Association of Official Analytical Chemists. *Official Methods of Analysis*, AOAC, Washington, D.C.

6. Evans, T.M., Seidleer, R. J., and LeChevallier, M.W. 1981. Impact of verification media and resuscitation on accuracy of the membrane filter total coliform enumeration technique. *Appl. Environ. Microbiol.*; 41:1144.

7. LeChevallier, M.W. and McFeters, G.A. 1984. Recent advances in coliform methodology for water analysis. *J. Evniron. Health.*; 47:5.

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