IFU

Instructions for Use

MEI AGAR

Cat. no. G124	mEI Agar, 15x60mm Plate, 11ml	10 plates/bag

INTENDED USE

Hardy Diagnostics mEI Agar is a selective culture medium recommended for use in the chromogenic detection and enumeration of enterococci in water by the single-step membrane filtration technique.

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

SUMMARY

Enterococci are found in the feces of humans and other warm-blooded animals. Although enterococci may be present without fecal pollution, enterococci are considered indicator organims in water samples. As such, the isolation of enterococci indicates the presence of fecal pollution and the potential for the presence of enteric pathogens.(1) It was found in epidemiological studies conducted by the EPA that the presence of enterococci had a higher correlation with swimming-associated gastroenteritis in fresh and marine water environments than fecal coliforms.(2) In 1986, the United States Environmental Protection Agency (U.S. EPA) recommended that both *Escherichia coli* and enterococci be used as bacterial indicators of recreational water quality.(3)

Levin, et al. developed a two-step membrane filter (MF) method to measure enterococci in fresh and marine recreational waters. Using mE Agar, the method required a 48-hour incubation and a transfer of the membrane to another substrate medium, Esculin Iron Agar, to differentiate enterococci. In 1997, the U.S. EPA improved the mE Agar formula by reducing the amount of triphenyltetrazolium chloride contained in the medium and also added the chromogen indoxyl-B-D-glucoside. The new medium, mEI Agar, was developed as a single-step procedure that does not require the transfer of the membrane to another substrate.(1-5) The development of a blue halo around colonies in 24 hours is confirmatory for he presence of enterococci. Utilizing this single-step MF procedure, various sample volumes or dilutions can be tested for the detection and enumeration of enterococci in potable, fresh, estuarine, marine and shellfish-growing waters.

Hardy Diagnostics mEI Agar utilizes the formula as described by Levin, et al. mEI Agar contains peptone that supplies nitrogen and cabon compounds. Sodium choride maintains osmotic equilibrium. Esculin is hydrolzed by enterococci to form esculetin and dextrose. Cycloheximide inhibits fungi. Sodium azide acts as a selective agent to inhibit gram-negative bacteria. Yeast extract provides trace elements, vitamins and amino acids. The addition of the chromogen indoxyl-B-D-glucoside results in the production of an insoluble indigo blue complex by B-D-glucosidase-positive enterococci, which diffuses into the surrounding medium, forming a blue halo around the colony. Agar is encorporated into the medium as a solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Yeast Extract	30.0gm
Sodium Chloride	15.0gm
Peptone	10.0gm
Esculin	1.0gm
Indoxyl-B-D-Glucoside	0.75gm
Sodium Azide	0.15gm
Cycloheximide	0.05gm
Agar	15.0gm

Final pH 7.1 +/- 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, 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 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

For for more detailed instructions on on sampling and membrane filtration procedures, consult Standard Methods for the Examination of Water and Wastewater .[7]

1. Collect and prepare water samples in accordance with recommended guidelines.
2. Select sample volumes to produce 20-60 colonies on the membrane filter.

3. After the sample has been filtered, aseptically remove the membrane filter from the filter base and roll it onto mEI Agar. **Avoid the formation of bubbles between the membrane and the agar surface.**
4. Invert the inoculated plate and incubate at 41 +/- 0.5ºC. for 24 +/- 2 hours.
5. After the incubation period, using an illuminated lens with 2-5x magnification, count and record the number of colonies with a blue halo.
6. Record the number of enterococci colonies present on the filter. Depending on the dilution counted, calculate the number of enterococci present per 100ml of sample.

Refer to the U.S. EPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[8]

INTERPRETATION OF RESULTS

Colonies that produce a blue halo may be presumptively identified as enterococci.

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.

Choose a water sample size that will result in 20-60 colonies per filter.

Minimize exposure of mEI Agar to light before and during incubation, as light may destroy the chromogen.

The US EPA has published a false-positive rate of 6.0% and a false-negative rate of 6.5%.[5] Colonies that produce a blue halo can be verified as enterococci by appropriate biochemical procedures in instances where required. Further biochemical testing may be required for evidence gathering or statistical evaluation of water sampling programs.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as membrane filters, membrane filtration apparatus, dilution blanks, illuminated lens, incubators, etc., as additional 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	
Enterococcus faecalis ATCC® 19433	MF	24hr	35°C	Aerobic	Growth; Blue halo
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition

* 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

mEI Agar should appear clear to very slightly opalescent and light to medium amber in color.

REFERENCES

1. U.S. Environmental Protection Agency, 1997. *Method 1600: Membrane filter test method for enterococci in water* . Publication EPA-821-R-97-004a. Office of Water, USEPA, Washington, D.C.

2. U.S. Environmental Protection Agency. 2000. *Improved enumeration methods for the recreational water quality indicators: enterococci and Escherichia coli* . Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.

3. U.S. Environmental Protection Agency. 1986. *Bacteriological ambient water quality criteria: availability* . FED. Reg. 51(45):8012.

4. Levin, Fisher and Cabelli. 1975. *Appl. Microbiol.* ; 30:66

5. U.S. Environmental Protection Agency. 2002. *Method 1600: Enterococci in water by membrane filtration using ebrane-enterococcus indoxyl-B-D-glucoside agar (mEI)* . Publication EPA821-R-02-022. USEPA Office of Water, Office of Science and Technology, USEPA, Washington, D.C.

6. Messer and Dufour. 1998. *Appl. Environ. Microbiol.* ; 64:678.

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

8. Bordner, Winter and Scarpino (ed.). 1978. *Microbiological Methods for monitoring the environment: Water and wastes* . Publication EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati., Ohio.

IFU-10856[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 2207D [D]