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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

CRITERION™ PRESENCE-ABSENCE BROTH

Cat. no. C6630	CRITERION™ Presence-Absence Broth	183gm
Cat. no. C6631	CRITERION™ Presence-Absence Broth	500gm
Cat. no. C6632	CRITERION™ Presence-Absence Broth	2kg
Cat. no. C6633	CRITERION™ Presence-Absence Broth	10kg
Cat. no. C6634	CRITERION™ Presence-Absence Broth	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Presence-Absence Broth, also known as P-A Broth, is used for detecting coliforms in treated water.

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

The Presence-Absence Broth test presumptively detects for coliforms in water. The test is a simple modification of the multiple-tube procedure.[4] One test sample, 100ml, is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms based on the presence or absence of lactose fermentation.[4] This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100ml water sample.[5-10]

Comparative studies with the membrane filter procedure indicate the Presence-Absence test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.[4] The Presence-Absence test is described in standard methods for water testing and by US EPA.[10]

FORMULA

Gram weight per liter (Single strength):	30.5gm/L
Lactose	7.46gm
Pancreatic Digest of Gelatin	5.0gm
Pancreatic Digest of Casein	4.92gm
Peptic Digest of Animal Tissue	4.91gm
Beef Extract	3.0gm
Sodium Chloride	2.46gm

Dipotassium Phosphate	1.35gm
Monopotassium Phosphate	1.35gm
Sodium Lauryl Sulfate	0.05gm
Bromcresol Purple	8.5mg

Final pH 6.8 +/- .02 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle 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 beige.

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 the following desired amounts of the dehydrated culture media in 1 liter of distilled or deionized water:

Single strength: 30.5gm/L
Double strength: 61.0gm/L
Triple strength: 91.5gm/L

2. Warm gently to dissolve completely.

3. Dispense 50ml amount into screw cap 250ml milk dilution bottles.

4. Autoclave at 121ºC. for 12 minutes, with the total autoclave time not to exceed 30 minutes.

5. Cool to room temperature.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

LIMITATIONS

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.

Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

The Presence-Absence test is only a presumptive test for coliforms.

Confirmation and differentiation of coliforms detected by the Presence-Absence test may be achieved by use of appropriate confirmatory media, incubation times and temperatures as outlined in appropriate references.

Extending the Presence-Absence test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

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 Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	18-48hr	35°C	Aerobic	Good growth; yellow color with or without gas production
Enterococcus faecalis ATCC® 29212	B	18-48hr	35°C	Aerobic	Moderate growth; slight yellow to purple color change
Pseudomonas aeruginosa ATCC® 27853	B	18-48hr	35°C	Aerobic	Poor to moderate growth; no color change

* 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™ Presence-Absence Broth powder should appear homogeneous, free-flowing, and beige in color. The prepared media should appear clear, slightly opalescent, and purple in color without significant precipitate.

REFERENCES

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

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

3. Eaton, A.D., et al. 1995. *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 19th ed. Washington, D.C.

4. Weiss, J.E. and C.A. Hunter. 1939. Simplified bacteriological examination of water. *J. Am. Water Works Assoc.*; 31:707-713.

5. Clark, J.A. 1968. The detection of various bacteria indicative of water pollution by a presence-absence procedure. *Can. J. Microbiol.*; 14:13-18.

6. Clark, J.A. and L.T. Vlassoff. 1973. Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence-absence test. *Health Lab. Sci.*; 10:163-172.

7. Clark, J.A. 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. *Can. J. Microbiol.*; 26:827-832.

8. Clark, J.A. 1980. The influence of increasing numbers of non-indicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. *Can. J. Microbiol.*; 26:827-832.

9. Clark, J.A., et al. 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. *Can. J. Microbiol.*; 28:1002-1013.

10. Federal Register. 1989. National primary drinking water regulations: total coliforms (including fecal coliforms and *E. coli*). *Fed. Regist.*; 54:27544-27568.

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

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