IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

LETHEEN BROTH, MODIFIED

Cat. no. K293	Letheen Broth, Modified, 16x125mm Tube, 9ml	20 tubes/box
Cat. no. U293	Letheen Broth, Modified, 8oz Wide Mouth Jar, 90ml	12 jars/box
Cat. no. U355	Letheen Broth, Modified with Tween® 80, 1.5%, 1L Polypropylene Bottle, 1000ml	10 bottles/box
Cat. no. U415	Letheen Broth, Modified, 500ml Polypropylene Bottle, 250ml	10 bottles/box

INTENDED USE

Hardy Diagnostics Letheen Broth, Modified is for use in microbial recovery from personal care products and for the neutralization of quaternary ammonium compounds in the testing of germicidal activity.[1,2]

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

SUMMARY

Quaternary ammonium compounds are molecules which contain a nitrogen atom bonded to four other atoms. Most quaternary ammonium compounds are organic compounds that have biological activity. These compounds often work well as disinfectants, offering bactericidal and bacteriostatic effects. Letheen Broth, Modified is used in the AOAC procedure for determining the effects of these bactericidal compounds.[1]

Letheen Broth, Modified is formulated as described in the U.S. FDA *Bacteriological Analytical Manual* and is recommended by the FDA for use in the microbiological examination of cosmetics; the FDA also mentions the use of this medium as a suitable enrichment broth for the identification of contaminants from non-sterile pharmaceutical products.[2,3] The medium contains beef extract and peptone which provide a nutrient rich source of vitamins, amino acids and nitrogen to support the growth of a variety of microorganisms. Yeast extract provides vitamins and co-factors which serve as additional sources of nitrogen and carbon required for bacterial growth. Letheen Broth, Modified also contains lecithin and Tween® 80. Quaternary ammonium compounds are neutralized by lecithin while phenolic disinfectants and hexachlorophene are neutralized by Tween® 80. Together, lecithin and Tween® 80 neutralize ethanol.[1]

FORMULA

Ingredients per liter of deionized water:*

Letheen Broth	25.7gm
Proteose Peptone No. 3	10.0gm
Casein Peptone	5.0gm
Yeast Extract	2.0gm

Sodium Bisulfite	0.1gm

In addition, Letheen Broth, Modified with Tween® 80, 1.5% (Cat. no. U355) contains:

Tween® 80	10.0gm

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration, 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

Consult AOAC and the U.S. FDA *Bacteriological Analytical Manual* for appropriate methods using Letheen Broth, Modified.[1-3]

INTERPRETATION OF RESULTS

Growth is indicated by turbidity in the medium. Subculture to a solid medium is necessary for confirmation and further identification. See appropriate references for the interpretation of growth in Letheen Broth, Modified.[1,3,4]

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.

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 Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Letheen Broth, Modified					
Staphylococcus aureus ATCC® 25923	J	24hr	30-35°C	Aerobic	Growth; turbidity
Escherichia coli ATCC® 25922	J	24hr	30-35°C	Aerobic	Growth; turbidity
Bacillus subtilis ATCC® 6633	J	24hr	30-35°C	Aerobic	Growth
Pseudomonas aeruginosa ATCC® 9027	J	24hr	30-35°C	Aerobic	Growth
Letheen Broth, Modified with Tween® 80, 1.5% (Cat. no. U355):**					
Candida albicans ATCC® 10231	J	1-3days	35°C	Aerobic	Growth; turbidity
Escherichia coli ATCC® 25922	J	1-3days	35°C	Aerobic	Growth; turbidity
Staphylococcus aureus ATCC® 25923	J	1-3days	35°C	Aerobic	Growth; turbidity

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

** Tested in accordance with USP.[4]

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

Letheen Broth, Modified and Letheen Broth, Modified with Tween® 80, 1.5% should appear slightly opalescent, light to medium amber in color, and may have slight precipitate.

REFERENCES

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

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

3. U.S. Food and Drug Administration. *Guide to Inspections of Microbiological Pharmaceutical Quality Control Laboratories*. Silver Springs, MD. http://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074914.htm

4.*United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

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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]