IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

MODIFIED LETHEEN AGAR

Cat. no. G221	Modified Letheen Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. W55	Modified Letheen Agar with Tween® 80, 1.5%, 15x100mm Plate, 28ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Modified Letheen Agar is used to determine the antimicrobial activity of quaternary ammonium compounds and in the microbial enumeration of cosmetics.

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 and have biological activity. These compounds often work well as disinfectants, offering bactericidal and bacteriostatic effects. Quaternary ammonium compounds are neutralized by lecithin, while phenolic disinfectants, formalin, and hexacholorphene are neutralized by Tween® 80. Together, lecithin and Tween® 80 neutralize ethanol.

Modified Letheen Agar is formulated as described by the U.S. FDA *Bacteriological Analytical Manual*.[1] Modified Letheen Agar is recommended by the FDA for use in the microbiological testing of cosmetics.[1,2] The medium contains beef extract and peptone to provide a nutrient rich medium supporting the growth of a wide variety of microorganisms. Modified Letheen Agar contains an elevated tryptone level to promote growth. Yeast extract provides vitamins and co-factors, and also serves as an additional source of nitrogen and carbon. Sodium chloride helps maintain osmotic balance. Modified Letheen Agar with Tween® 80, 1.5% contains additional Tween® 80 to further neutralize and reduce the surface tension of sampled materials.

FORMULA

Ingredients per liter of deionized water:*

Letheen Agar	32.0gm
Proteose Peptone No. 3	10.0gm
Tryptone	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Sodium Bisulfite	0.1gm
Agar	5.0gm

In addition, Modified Letheen Agar with Tween® 80, 1.5% contains:

Tween® 80	8.0gm

Final pH 7.2 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

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 listed references and regulatory guidelines for best practices.[1-6]

1. Prepare and dilute samples in Modified Letheen Broth in accordance with established guidelines.

2. Using the spread plate technique, inoculate in duplicate 0.1ml of the diluted samples onto Modified Letheen Agar, Potato Dextrose Agar (or Malt Extract Agar) containing chlortetracycline, Baird-Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Modified Letheen Agar plates.

3. Incubate one set of Modified Letheen Agar plates at 30 +/- 2ºC. for 48 hours and the other set at 35 +/- 2ºC. for 2-4 days. Incubate the Potato Dextrose Agar (or Malt Extract Agar) plates at 30 +/- 2ºC. for 7 days, and the Baird-Parker Agar (or Vogel-Johnson Agar) plates, if inoculated, at 35 +/- 2ºC. for 48 hours.

4. Incubate the diluted samples from step 1 at 35 +/- 2.0ºC. for 7 days. Subculture enriched samples onto Modified Letheen Agar only if there is no growth on the primary Modified Letheen Agar plates.

INTERPRETATION OF RESULTS

See listed references for the interpretation of growth using Modified Letheen Agar.

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	
Modified Letheen Agar (Cat. no. G221):**					
Staphylococcus aureus ATCC® 25923		24hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	J	24hr	35°C	Aerobic	Growth
Modified Letheen Agar with Tween® 80, 1.5% (Cat. no. W55):**					
Candida albicans ATCC® 10231	J	1-3days	35°C	Aerobic	Growth
Staphylococcus aureus ATCC® 25923	J	1-3days	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	J	1-3days	35°C	Aerobic	Growth
Pseudomonas aeruginosa ATCC® 9027	J	1-3days	35°C	Aerobic	Growth
Bacillus subtilis ATCC® 6633	J	24hrs	35°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	G	1-5days	25°C	Aerobic	Growth
Candida albicans ATCC® 10231	J	1-5days	25°C	Aerobic	Growth

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

** Tested in accordance with USP.[6]

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

Modified Letheen Agar and Modified Letheen Agar with Tween® 80, 1.5% should appear clear, and light amber in color.

REFERENCES

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

2. Orth, D.S. 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc. New York, NY.

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

4. American Public Health Association Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*. APHA, Washington, D.C.

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

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

IFU-10582[B]

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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.