IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

LETHEEN MEDIA

Cat. no. G42	Letheen Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. Q18	Letheen Agar, 20x125mm Tube, 18ml Deep	20 tubes/box
Cat. no. K106	Letheen Broth, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. U39	Letheen Broth, 8oz Wide Mouth Jar, 90ml	12 jars/box
Cat. no. U40	Letheen Broth, 180ml Wide Mouth Jar, 99ml	12 jars/box
Cat. no. U371	Letheen Broth, 1L polypropylene bottle, 1000ml	10 bottles/box

INTENDED USE

Hardy Diagnostics Letheen Agar is used for evaluating the bactericidal activity of quaternary ammonium compounds. Letheen Broth is used for determining the phenol coefficient of cationic surface-active materials.

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 with four other atoms bonded to it. Most quaternary ammonium compounds are organic compounds and have biological activity. These compounds often work well as disinfectants, offering bactericidal and bacteriostatic effects.

Letheen Media is formulated according to the guidelines set by the AOAC for the determination of phenol coefficient of quaternary ammonium compounds.[1] The medium contains beef extract and peptone to provide a nutrient rich medium supporting the growth of a variety of microorganisms. Quaternary ammonia compounds are neutralized by lecithin while phenolic disinfectants and hexachlorophene are neutralized by Tween® 80. Together, lecithin and Tween® 80 neutralize ethanol.

Letheen Broth is used in the AOAC for use with disinfectants containing cationic surface active materials.[1] Letheen Agar and Letheen Broth are specified for use by the American Society for Testing Materials (ASTM) in the Standard Test Method for Preservatives in Water Containing Cosmetics.[4]

Letheen Broth is available in a 99ml fill volume for preparation of 1:100 test dilutions.

FORMULA

Ingredients per liter of deionized water:*

Letheen Agar	
Tween® 80	7.0gm

Pancreatic Digest of Casein	5.0gm
Beef Extract	3.0gm
Dextrose	1.0gm
Lecithin	1.0gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC.

Letheen Broth	
Peptic Digest of Animal Tissue	10.0gm
Tween® 80	5.0gm
Sodium Chloride	5.0gm
Beef Extract	5.0gm
Lecithin	0.7gm

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store Letheen Media 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

Allow media to warm to room temperature before inoculation.

Please consult AOAC for methods using Letheen Media.[1]

The Letheen Agar, Deep (Cat. no. Q18) can be used for plate counts using either the spread plate method or pour plate method. See listed references for more information.[2,3]

Spread Plate Method:

1. Warm media to room temperature.

3. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

4. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.

5. Spread the dilution evenly over the surface of the medium.

6. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.

7. Incubate plates aerobically for 48 +/- 2.0 hours at 35ºC.

Pour Plate Method:

1. Melt agar by placing in a boiling waterbath until liquified.

2. Cool media to 45-50ºC. Maintain in a 45-50º waterbath until ready to pour.

3. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

4. Place a 1ml inoculation into a sterile petri plate.

5. Aseptically pour approximately 18ml of the cooled media (45-50ºC.) over the inoculum. Carefully swirl the plate to mix the inoculum evenly.

6. Allow to solidify.

7. Incubate plates aerobically for 48 +/- 2.0 hours at 35ºC.

INTERPRETATION OF RESULTS

See listed references for the interpretation of growth in Letheen Media.[1-4]

Plate Counts:

Following incubation, examine the plates for growth. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample (take into account the dilution factor). If duplicate plates were set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on this medium.[1-4]

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, 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	
Letheen Agar					
Staphylococcus aureus ATCC® 25923	A	24-48hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	24-48hr	35°C	Aerobic	Growth
Salmonella typhimurium ATCC® 14028	A	24-48hr	35°C	Aerobic	Growth
Letheen Broth					
Staphylococcus aureus ATCC® 25923	A	18-48hr	35°C	Aerobic	Growth; turbidity
Escherichia coli ATCC® 25922	A	18-48hr	35°C	Aerobic	Growth; turbidity
Salmonella typhimurium ATCC® 14028	A	18-48hr	35°C	Aerobic	Growth; turbidity

* 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

- Letheen Agar should appear clear, with a slight opalescence, and light amber in color.
- Letheen Broth should appear clear, with a slight opalescence, ans light to medium amber in color.

Staphylococcus aureus (ATCC® 25923) colonies growing on Letheen Agar (Cat. no. G42). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) colonies growing on Letheen Agar (Cat. no. G42). Incubated aerobically for 24 hours at 35ºC.

Uninoculated plate of Letheen Agar (Cat. no. G42).

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.* Arlington, VA
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

3. The Official Compendia of Standards. *USP-NF* . United States Pharmacopeial Convention, Rockville, MD.

4. *American Society for Testing Materials* . Standard test method for preservatives in water-containing cosmetics. 1991. E: 640-78. Annual Book of ASTM Standards, Philadelphia, PA.

IFU-10523[A]

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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]