IFU

Instructions for Use

LOEFFLER MEDIUM

Cat. no. L28	Loeffler Medium, 16x100mm Tube, 5.5ml Slant	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Loeffler Medium is recommended for the cultivation of *Corynebacterium diphtheriae*.

This medium is also useful in demonstrating proteolysis and pigment production of various microorganisms.

SUMMARY

Hardy Diagnostics Loeffler Medium is a modification of the original formula developed by Loeffler in 1887.[6-8] The medium contains horse serum, beef extract, dextrose and proteose peptones which together supply the complex nitrogenous substances and nutrients necessary to support the growth of *Corynebacterium diphtheriae*. Sodium chloride is added to supply essential ions.

The medium enhances the development of metachromatic granules as seen in methylene blue stains. Formation of the granules demonstrates the characteristic cellular morphology of *C. diphtheriae*.

Loeffler Medium is also useful for demonstrating pigment production and in determining the proteolytic activity of various microorganisms.

FORMULA

Ingredients per liter of deionized water:*

Proteose Peptone	1.5gm
Dextrose	1.25gm
Sodium Chloride	1.25gm
Beef Extract	0.75gm
Horse Serum	750.0ml

Final pH 7.6 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees 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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

For Isolation of Corynebacterium diphtheria:

Method of Use:

1. Prior to inoculation, allow the medium to equilibrate to room temperature.

2. Using a fishtail motion, directly inoculate specimen swab onto the medium.

3. Incubate aerobically at 35ºC. for up to 4 days.

4. Observe daily for typical colonial morphology of corynebacteria.

5. Perform methylene blue stain to check for the presence of metachromatic granules and appearance suggestive of chinese-letter formation of cells.

6. Definitive identification of *C. diphtheriae* is made by performing biochemical and toxigenicity tests.

For Detection of Proteolysis of Aerobic Microorganisms

1. Inoculate medium with isolated colonies of the organism in question.

2. Incubate aerobically at 35ºC. for 3-4 days.

3. Observe for typical colonial morphology.

F **or Detection of Proteolysis of Anaerobic Microorganisms**

1. Inoculate medium with isolated colonies of the organism in question.

2. Incubate anaerobically at 35ºC. for 3-4 days **or** overlay the inoculated slant with Thioglycollate Broth just prior to incubation, tighten cap and incubate aerobically.

3. Observe for typical colonial morphology.

INTERPRETATION OF RESULTS

Growth of *Cornyebacterium* species on Loeffler Medium appear as minute, and cream colored colonies with slightly raised centers.

Cornyebacterium species reveal metachromatic granules and appearance suggestive of chinese-letter formation in methylene blue stain.

Proteolysis is indicated by the appearance of colonies surrounded by a small crater of liquefied medium or liquefaction of the slant with the production of a putrid odor.

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.

It is recommended that selective and non-selective media be inoculated in parallel to Loeffler Medium for isolating *C. diphtheriae*; Potassium Tellurite Cystine Agar and Blood Agar are recommend for enhanced recovery.[5]

To optimize recovery of *C. diphtheriae*, a nasopharyngeal and throat specimen should be obtained upon specimen collection.

Variation in microscopic morphology may vary from lot to lot of Loeffler Medium.

Gram-positive microorganisms other than *Corynebacterium* may produce metachromatic granules when grown on Loeffler Medium.

Detection of proteolysis by some microorganisms may require incubation periods beyond the recommended four days.

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	

Corynebacterium diphtheriae ATCC® 13812	A	72-96hr	35°C	Aerobic	Growth; metachromatic granules seen in methylene blue stain

* 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

Loeffler Medium should appear opaque, and gray-white in color.

Corynebacterium diphtheriae (ATCC® 13812) colonies growing on Loeffler Medium (Cat. no. L28). Incubated aerobically for 72 hours at 35ºC.

Uninoculated tube of Loeffler Medium (Cat. no. L28).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Loeffler, F. 1897. *Zentralb. Bakteriol. Parasitenkd*; 2:102-106.

7. Petran, E.L. and Perry, C.A. 1929. *J. Lab. Clin. Med.*; 25:71-78.

8. Buck, T.C. 1949. *J. Lab. Clin. Med.*; 34:582-582.

ATCC is a registered trademark of the American Type Culture Collection.

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