
LOEFFLER'S MEDIUM

INTENDED USE

Remel Loeffler's Medium is a solid medium recommended for use in qualitative procedures for the cultivation of *Corynebacterium diphtheriae* from clinical specimens.

SUMMARY AND EXPLANATION

In 1887, Loeffler developed a medium containing horse serum, beef heart infusion, and dextrose for cultivating corynebacteria.¹ Buck and Perry et al. described modifications of Loeffler's medium.^{2,3} The current formulation of Loeffler's Medium is an incorporation of previous formulas developed to elicit the formation of metachromatic granules, which are characteristic of *C. diphtheriae*.⁴ Loeffler's Medium is also used for cultivation of pathogenic strains of *C. diphtheriae* to enhance microscopic and colonial morphology, chromogenesis, and natural virulence. This medium will often restore these properties to strains which have lost them due to prolonged incubation and repeated subculture.

PRINCIPLE

Casein peptone and beef heart infusion supply nitrogen, amino acids, and peptides necessary for the growth of corynebacteria. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Dextrose is a fermentable carbon energy source and yeast extract supplies vitamins. Horse serum is a source of protein used in the metabolism of corynebacteria and allows for the determination of proteolytic activity.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone.....	10.0 g	Beef Heart Infusion.....	2.0 g
Sodium Chloride.....	5.0 g	Horse Serum	750.0 ml
Yeast Extract.....	5.0 g	Agar.....	15.0 g
Dextrose.....	2.5 g	Demineralized Water.....	250.0 ml

pH 7.6 + 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate the specimen as soon as possible after it is received in the laboratory.
2. For isolation of *Corynebacterium* spp. from potentially contaminated specimens, inoculate a selective medium (e.g., Cystine Tellurite Blood Agar) along with Loeffler's Medium.
3. Incubate aerobically with caps loosened at 33-37°C for up to 4 days. Do not incubate in CO₂.
4. Examine cultures after 24 hours for minute, cream-colored colonies with slightly raised centers, characteristic of *C. diphtheriae*.
5. Prepare smears and stain with Loeffler's Methylene Blue stain (REF R40083) following established laboratory procedures. Examine for the presence of dark-blue metachromatic granules.
6. Subculture colonies that exhibit typical morphology to a blood agar plate for additional testing. Further cultural, biochemical, and toxigenicity tests must be performed for differentiation and identification of *Corynebacterium* spp.⁴ Consult appropriate references for further instructions.⁵⁻⁷

QUALITY CONTROL

All lot numbers of Loeffler's Medium have been tested using the following quality control organism and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Corynebacterium diphtheriae ATCC® 13812

INCUBATION

Aerobic, up to 72 h @ 33-37°C

RESULTS

Growth

LIMITATIONS

1. Other gram-positive organisms may produce metachromatic granules when grown on Loeffler's Medium.⁴
2. *C. diphtheriae* must be a toxigenic strain to be diagnostic of diphtheria; non-toxigenic strains will also grow on this medium.⁵

BIBLIOGRAPHY

1. Loeffler, F. 1897. Zentralbl. Bakteriell. Parasitenkd. 2:102-106.
2. Petran, E.L. and C.A. Perry. 1939. J. Lab. Clin. Med. 25:71-78.
3. Buck, T.C. 1949. J. Lab. Clin. Med. 34: 582-583.
4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
5. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.
6. Winn, W., S. Allen, J. William, E. Koneman, G. Procop, P. Schreckenberger, and G. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins, Baltimore, MD.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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