Simplified Henry Technique for Initial Recognition of Listeria Colonies

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The Henry oblique transmitted-light viewing technique was modified to provide a more precise, convenient, and familiar manner with which to read (score) and recognize colonies of listeriae by their distinct bluish cast. The simplified technique involved illuminating each colony directly with a high-intensity lamp while viewing it with a hand lens at a precise angle in place of a scanning light microscope.

For over 40 years, the Henry oblique transillumination (HOT) technique (6, 8) has been used for the initial recognition of Listeria colonies that appear with a distinctive bluish cast, facilitating their selection in the midst of numerous other colonies (15). It is currently included in two protocols (11, 13) devised by U.S. regulatory agencies for the detection of food-borne listeriae; other laboratories have reported satisfactory results with the technique (5, 14). However, other investigators (1, 7, 16) have reported that certain non-Listeria organisms, especially streptococcal colonies, exhibit a similar bluish cast. A likely explanation for this observation is the difficulty in setting a precise angle with which to obliquely illuminate the object with a mirror. Seeleiger and Jones (15) have emphasized the importance of the angle of light with the HOT technique. Another disadvantage with the technique is its cumbersome procedure of viewing uncovered agar plates right side up instead of viewing covered plates bottom side up; the latter would allow scoring for typical colonies with the use of a marking pen. Consequently, investigators have explored alternative diagnostic properties, such as tellurite reduction (2) or dark color resulting from the reaction of ammonium ferric citrate with the hydrolytic product of esculin (3, 17). However, these properties lack specificity, a problem that especially occurs with streptococci that manage to grow on the various Listeria plating media (1, 4, 12, 17). The present study reports a successful modification of the HOT technique which provides a more precise, convenient, and familiar manner with which to read and recognize colonies of listeriae on a recently developed Listeria plating medium (LCA; 9). LCA consists of brain heart infusion agar (BHIA; Difco Laboratories) containing the following selective agents: 0.5% LiCl, 1.0% glycerine anhydride, and 50 mg of ceftazidime panhydrate (Glaxo Pharmaceuticals, Ltd.) per liter.

(Results of this study were presented at the Annual Meeting of the Society for Industrial Microbiology in Seattle, Wash., 13 to 18 August 1989 [Abstr. Annu. Meet. Soc. Ind. Microbiol. 1989, P44, p. 86]).

In reassessing the HOT technique (Fig. 1), it became apparent that the focus on the angle of reflected light (β = 45°) was misdirected. In essence, the technique consists of viewing the object at point B (the colony) at a right angle (90°) from point A and illuminating it from point C with white light reflected by a mirror at 45°. Thus, the angle of concern is actually α (designated as the angle of transillumination), whose value (135°) is the sum of 45° and 90°. It also became apparent that neither a mirror for illumination of the object nor a scanning light microscope was necessary for viewing a colony with oblique lighting. Consequently, a simplified HOT (SHOT) technique was developed (Fig. 2). For ease of viewing and counting (scoring) of suspect colonies, each agar plate was examined by placing it bottom side up on a transparent platform that was tilted towards the viewer at 45° and illuminated directly from below with a high-intensity lamp at a right angle (90°) to the bench top. Each colony on the agar plate was scanned with a 5× magnifying hand lens attached to a tube that was clamped at 45° with the aid of a set square, thereby attaining the angle of transillumination of 135°. The agar plates were freed of water condensate to reduce distortion.

The SHOT technique was evaluated with 59 strains of listeriae consisting of Listeria monocytogenes (24 strains) of serotypes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 6b, and 7; L. seeligeri (7 strains); L. ivanovii (2 strains); L. welshimeri (10 strains); L. innocua (14 strains); L. grayi (1 strain); and L. murrayi (1 strain). The nonlisterial strains used included the following: Brochothrix thermosphacta, Corynebacterium aquaticum, Citrobacter freundii, Enterobacter cloacae, Jonesia dentritificans, Klebsiella pneumoniae, Kurthia zopfii, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, S. epidermidis, and Streptococcus faecalis. Most of the cultures were kindly provided by investigators who have published works on listeriae.

All the listeriae on LCA plates that were streak inoculated to obtain well-isolated colonies after 40 h at 30°C exhibited the bluish cast. The large, well-isolated colonies showed the bluish hue primarily on the rim, whereas the bluish hue was uniform among the small colonies on the crowded sections of the agar plates. A distinct bluish hue was readily discerned when the thick, opaque center portion of a large colony was streaked into a thin layer. This distinctive hue was practically obliterated when the listeriae were grown on BHIA containing 4% NaCl or 1.5% LiCl.

Among the nonlisterial strains growing on BHIA, only C. aquaticum, J. dentritificans, A. cloacae, P. aeruginosa, and S. marcescens appeared to have a bluish hue similar to that of the listeriae. LCA was completely inhibitory for these strains, with the exception of C. aquaticum, which grew as pin-point colonies, in marked contrast to the large colonies of listeriae.

Colonies with atypical iridescence or morphology were picked during a routine screening for listeriae by direct plating on LCA of retail-level foods (brie, camembert, yogurt, sliced chicken luncheon meat, and pork sausage). These samples turned out to be negative for listeriae (<100
CFU/g). The colonies consisted of 7 sporeforming Bacillus strains, 5 yeasts, 13 catalase-negative cocci (streptococci), and 11 catalase-positive cocci (staphylococci). The lactic acid bacteria from yogurt that formed tiny colonies on BHIA plates appeared reddish when viewed by the SHOT technique; No colonies with the distinctive bluish cast of Listeria colonies were encountered.

The efficacy of the SHOT technique was also verified with five frozen retail-level food samples that were recently reported to be naturally contaminated with moderate levels of L. monocytogenes (>100 CFU/g) (9). When these samples were plated on LCA, all but one (precooked sliced beef) were positive for L. monocytogenes at levels similar to those in the previous report (9). The suspect colonies were easily recognized by their bluish cast when viewed by the SHOT technique. Subsequently, representative colonies were all definitively identified as L. monocytogenes in less than 8 h with the use of a recently developed diagnostic scheme (10). This scheme involved subjecting each representative suspect colony to a series of accelerated conventional tests for hemolysis and sugar fermentations as well as phase-contrast microscopy, catalase production, and the KOH viscosity test. All the colonies that exhibited the typical bluish cast were L. monocytogenes.

The results of this study demonstrate that the SHOT technique is a convenient and effective procedure for the initial recognition of colonies of listeriae recovered on LCA from foods before definitive identification at the species level. Problems with false-positives or false-negatives were not encountered.

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LITERATURE CITED