Limitations of Lysine-Iron-Cystine-Neutral Red Broth in the Presumptive Identification of Salmonella

J. Y. D'Aoust
Bureau of Microbial Hazards, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

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Lysine-iron-cystine-neutral red broth performed satisfactorily in the presumptive identification of Salmonella in preenriched food and animal feed samples enriched in tetrathionate-brilliant green broth. Homologous results from selenite-cystine enrichment broths yielded unacceptably high numbers of false negative reactions.

Lysine-iron-cystine-neutral red (LICNR) broth has been shown to be an effective single-step enrichment medium for the presumptive identification of Salmonella in raw milk, nonfat dry milk, and cheese (5). Salmonella cultures decolorize the medium from red to yellow and usually form a black, iron sulfide precipitate; related enteric bacteria do not change the color of the medium. After an additional 24 h of incubation, the alkaline reaction of H₂S-negative strains of Salmonella permit differentiation of these strains from Escherichia coli and Klebsiella species. Lactose-positive salmonellae decolorize the medium and form a black precipitate. Further studies found the broth to be an effective single-step enrichment in the analysis of nonfat dry milk for Salmonella by immunofluorescent techniques (8). Use of the presumptive LICNR broth for rapid enrichment of foods other than milk and cheese, food ingredients, and feed materials met with limited success due to interference of certain foods with the color reactions of the medium (6); nonetheless, performance of the broth markedly improved when used for secondary enrichment. LICNR broth was recently adapted to a pour plate agar medium for the direct enumeration of Salmonella in artificially contaminated dairy products (9); typical salmonellae form black, lenticular colonies surrounded by a purple zone within 24 to 48 h of incubation. The present study evaluates the performance of LICNR broth as an alternate to conventional plating media for the presumptive identification of Salmonella.

Chili powder was artificially contaminated with a log-phase broth culture of S. typhimurium, rapidly frozen in liquid nitrogen, and dried under vacuum. Nonfat dry milk was seeded with an H₂S-negative biotype of S. senftenberg according to the method of Edel and Kampelmacher (2). Other foods tested in this study were naturally contaminated at levels of 0.3 to 46.0 salmonellae per 100 g.

Most food samples prepared for a three-tube most-probable-number estimation of Salmonella contamination were preenriched in nutrient broth at 37°C for 18 to 24 h; chocolate and cocoa powder samples were prepared in 10% (wt/vol) skim milk powder, and nonfat dry milk was reconstituted in sterile distilled water containing 0.002% (wt/vol) brilliant green dye. Portions of preenriched cultures were transferred into tetrathionate-brilliant green and selenite-cystine broths and incubated for 18 to 24 h at 43 and 37°C, respectively. Each enrichment broth was streaked onto bismuth sulfite and brilliant green sulfasa agars, and 1.0 ml from each enrichment broth was also inoculated into 9.0 ml of LICNR broth; all plates and LICNR broths were incubated at 37°C for 24 h. Presumptive Salmonella colonies on the selective plating media were screened biochemically on triple sugar-iron and lysine-iron agars and confirmed serologically. Presumptively positive LICNR tubes were identified according to reactions described previously (5). Bacteriological media were obtained from Difco Laboratories (Detroit, Mich.), who distributes LICNR broth as Special Broth no. 1 C.S. (C-026-17).

In the LICNR short method (6), food samples were directly enriched in tetrathionate broth at 41°C for 20 h. Portions (1.0 ml) of enriched cultures were transferred to tubes of LICNR broth and incubated at 37°C for 24 h. Positive LICNR tubes were confirmed by streaking onto bismuth sulfite and brilliant green sulfasa agars, and presumptive Salmonella isolates were screened as described previously.

LICNR broth performed satisfactorily in combination with the tetrathionate-brilliant green enrichment medium, eliciting a single false negative reaction with cocoa powder; animal feeds were primarily responsible for the 13 false positive out of 99 tube reactions (Table 1). In addi-
tion to the usual development of strong color reactions, the LICNR medium detected H$_2$S gas production in a strain of S. senftenberg in nonfat dry milk documented to be H$_2$S-negative on triple sugar-iron. In contrast, the selenite-cystine–LICNR combination proved to be entirely unreliable, detecting only 3 out of 39 tube reactions found to be positive by conventional methods. Reduction of the selenite-cystine inoculum size reportedly eliminates false negative reactions (D. A. Hoben, personal communication). Recovery of salmonellae with the LICNR short method was also unsatisfactory, yielding 24 false positive reactions. Attempts to isolate bacterial strains responsible for false positive reactions were unsuccessful. Our results support an earlier suggestion (6) that synergy between microorganisms, rather than single strains, was responsible for the observed erroneous reactions. Although a novobiocin-supplemented LICNR broth eliminated false positive reactions (6), the antibiotic was not used in our studies because its effect on the recovery of Salmonella is poorly documented.

Our findings suggest that current Salmonella methodology recommending the use of tetra- thionate-brilliant green and selenite-cystine enrichment broths in parallel (1, 3, 4, 7, 10) cannot accommodate the LICNR broth as an alternate to differential plating media, owing to the unacceptable level of false negative reactions obtained with the selenite-cystine–LICNR combination. Although the performance of the tetra- thionate-brilliant green–LICNR combination was significantly better, the 13/99 (13%) false positive reactions (Table 1) and the occurrence of a false negative reaction with cocoa powder warrant caution in the use of this analytical scheme for the rapid screening of samples not likely to contain Salmonella.

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### LITERATURE CITED


