Motility-Indole-Lysine-Sulfide Medium

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A medium designed for the detection of motility, indole, lysine decarboxylase and deaminase reactions, and H₂S production was devised and evaluated. Results, using 157 strains of enteric pathogens, were in agreement with reference methods. When 300 isolates from fecal cultures were screened using this medium, *Shigella* was easily differentiated from *Escherichia* and more of the *Proteus* species, especially *P. morganii*, could be eliminated from further study.

Lysine iron agar (LIA) was first described by Edwards and Fife (3) in 1961. Since that time Edwards and Ewing (2) have recommended the use of LIA in combination with triple sugar iron agar (TSI) for subculturing all suspect colonies observed on primary plating media of stool cultures for *Salmonella-Shigella*. Although most stool isolates screened with TSI and LIA require no further biochemical testing to determine the presence or absence of a pathogen, for some isolates it is necessary to determine motility, indole production, and urease activity as a minimum of additional biochemical tests.

The purpose of this study was to develop and evaluate a combination medium (motility-indole-lysine-sulfide [MILS]) in which lysine decarboxylase and deaminase reactions could be detected in conjunction with tests for indole, H₂S production, and motility in a single tube. The following formula was used: 9.0 g of decarboxylase medium base (Difco no. 0872), 5.0 g of peptone (Difco), 10.0 g of tryptone (Difco), 2.0 g of agar, 0.5 g of ferric ammonium citrate, 0.04 g of sodium thiosulfate, 10 g of L-lysine monohydrochloride (Eastman Kodak Co., Rochester, N.Y.) and 1,000 ml of distilled water. The medium was heated to dissolve the agar and was dispensed in 5-ml amounts into tubes (13 by 100 mm), capped, and autoclaved at 121 C for 15 min. The final pH of the medium before autoclaving was 6.6 ± 0.1. For use, each isolate was inoculated into the medium by stabbing with a straight wire to the bottom of the tube. After 18 to 24 h of incubation at 35 C the reactions were read; indole was read last since the addition of Kovacs solution interfered with other reactions. Motility was indicated by generalized turbidity or growth extending from the line of inoculation; nonmotile organisms grew only along the line of inoculation. Lysine decar-

boxylase production was indicated by a purple color throughout the tube; the butt was yellow with a band of purple at the top for organisms which were lysine decarboxylase negative. *Proteus* and *Providencia* species produced a band of red at the top of the medium and a yellow butt. The production of H₂S was indicated by blackening at the top of the medium or blackening at the line of inoculation. Three to four drops of Kovacs reagent were added to the medium to detect indole production; the development of a red-to-pink color indicated that indole had been produced, and a bright yellow color indicated a negative reaction.

Strains of *Proteus*, *Providencia*, *Citrobacter*, *Escherichia*, *Serratia*, *Klebsiella*, and *Enterobacter* were tested on MILS. The reactions for these genera were in agreement with the results of testing with LIA (Difco) and motility-indole-ornithine medium (1) (Difco) with the exception of *Proteus morganii*; MILS was a superior medium for this organism. For example, when 19 strains of *P. morganii* were tested, only 3 strains produced a typical red slant on LIA; in contrast, 13 of the 19 strains produced a red band in MILS.

Stock strains including 126 *Salmonella*, 20 *Arizona*, 11 *Edwardsiella*, and 46 *Shigella* were tested on MILS. The reactions on MILS were in agreement with reference methods (2), although for two strains of *E. tarda* the blackening due to H₂S production was so intense the lysine decarboxylase reaction was difficult to read.

After the initial evaluation of MILS a field trial was performed at the University of Minnesota. Two hundred colonies from stool cultures were subcultured to TSI, LIA, and MILS. Comparable results were obtained on LIA and MILS for 184 of the isolates, and superior results were

obtained with MILS for the remaining 16 isolates. Four of these 16 isolates showed questionable lysine decarboxylase reactions on LIA; these were clearly positive on MILS. Twelve of the isolates produced a band of red at the top of the medium characteristic of *Proteus* species; no red color was seen on LIA.

At the University of Colorado a similar field trial was performed on 100 fecal isolates using MILS and comparing the reactions on this medium with those on TSI and motility-indole-lysine medium (MIL) (4), a medium developed at the University of Colorado. In this trial all of the isolates were identified using reference methods (2). The reactions on MILS and MIL were in agreement with the following exceptions: 1 of 31 *E. coli* was indole negative on MILS and positive on MIL and the reference method, and 2 of 5 *P. morganii* produced a red band at the top of MILS and no red color in MIL. Since H_2S production cannot be determined with MIL, TSI was used for comparison. Five of thirteen *Citrobacter freundii* and two of three *P. mirabilis* demonstrated H_2S production on TSI but were negative on MILS. The single isolate of *P. vulgaris* in this group produced so much H_2S on MILS that the decarboxylase reaction was difficult to read.

MILS has been used at the University of Minnesota in the Clinical Microbiology Labora-

tory for the past 6 months. Since five tests can be determined in a single tube, fewer subcultures are required to verify the presence or absence of pathogens. The detection of motility has been an aid in the rapid differentiation of most *E. coli* from *Shigella* species. The increased sensitivity of MILS to detect *P. morganii* has proven particularly helpful in screening stool cultures for *Salmonella* and *Shigella* since these isolates can be discarded without further subcultures. In both the initial studies and in routine use, MILS medium provided more useful information than did LIA in the evaluation of organisms found in fecal cultures.

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