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Evaluation of Urea-Motility-Indole Medium for Recognition and Differentiation of *Salmonella* and *Shigella* Species in Stool Cultures

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A semisolid urea-motility-indole medium designed for detection in *Enterobacteriaceae* of urease activity, motility, and indole production in one tube was prepared and evaluated. The formulation of the medium was similar to that of Christensen urea agar, but the agar concentration was 0.2%, and 1% tryptone was added. Results with 687 strains of *Enterobacteriaceae* were the same as those obtained with standard test media (98% overall agreement). The urea-motility-indole medium was also used in combination with Kligler iron agar for the recognition and differentiation of *Salmonella* and *Shigella* species from colonies picked from plating media in fecal cultures. This combination was compared with the combination of Kligler iron agar and lysine iron agar with 507 strains of non-lactose-fermenting *Enterobacteriaceae*. Although both combinations enabled the presumptive recognition and differentiation of *Salmonella* and *Shigella* species, an analysis of data indicated that the combination of Kligler iron agar and urea-motility-indole medium performed better than the combination of Kligler iron agar and lysine iron agar in detecting *Salmonella* and *Shigella* species.

The detection of *Salmonella* and *Shigella* species in fecal cultures is an important part of the workload in any laboratory of clinical microbiology. In practice this means the processing or screening of suspicious colonies isolated from primary plating media. For facilitating this differentiation many differential media have been devised, among which the most widely used is Kligler iron agar (KIA) or triple sugar iron agar, used in conjunction with lysine iron agar (LIA) (4-8, 14, 16). Another recently proposed approach is the use, in conjunction with KIA (or triple sugar iron agar), of a motility-indole-lysine medium (7, 11).

Despite the general usefulness of these media, the detection of urease activity remains fundamental in the detection of bacterial enteric pathogens in fecal cultures as it provides the early detection and exclusion of *Proteus*, which is one of the genera most frequently picked from primary plating media (9). Thus, we have prepared and evaluated a urea-motility-indole (UMI) medium that enables the quick determination of urease activity, motility, and indole production in one tube, to be used in conjunction with KIA (or triple sugar iron agar) to process colonies resembling enteric pathogens in routine stool cultures.

In this paper we evaluate the accuracy of the UMI medium in the detection of urease activity, motility, and indole production in *Enterobacteriaceae* and compare the results obtained with the UMI medium with the results obtained with standard test media. We also compare the ability of KIA-UMI and KIA-LIA (4) combinations of media to recognize, as *Salmonella* or *Shigella* species, suspicious colonies picked from primary plates in fecal cultures.

MATERIALS AND METHODS

UMI medium. For facilitating the preparation of UMI medium, urea agar base was used as the basic component (Difco Laboratories, Detroit, Mich.) The medium was prepared in the same way as Christensen urea agar (1, 2), although the final agar concentration was lowered to 2 g/liter, and 10 g of tryptone (Difco) per liter was added. The final pH was adjusted to 6.8 ± 0.2, if necessary, with sterile 1 M HCl or 1 M NaOH. After preparation and before solidification, the medium was dispensed under aseptic conditions in 3-ml amounts into tubes (8 by 80 mm).

Standard media and LIA. The detection of urease activity, motility, and indole production was performed by the methods described by Edwards and Ewing (4). All of the media were prepared from commercially available dehydrated stocks (Difco). LIA (5) was obtained as the dehydrated product from Oxoid Ltd., Basingstoke, Hampshire, England (10).

Bacteria. For evaluating the KIA-UMI and KIA-LIA combinations, a total of 507 strains of *Enterobacteriaceae* that do not ferment lactose in 18 to 24 h were tested. These included 60 strains of *Escherichia coli*, 23 of *Shigella flexneri*, 18 of *Shigella sonnei*, 2 of *Shigella boydii*, 6 of *Enterobacter aerogenes*, 3 of

Enterobacter cloacae, 40 of *Serratia marcescens*, 5 of *Serratia liquefaciens*, 40 of *Salmonella enteritidis*, 8 of *Salmonella typhi*, 5 of *Citrobacter freundii*, 218 of *Proteus mirabilis*, 7 of *Proteus vulgaris*, 62 of *Proteus morganii*, 3 of *Proteus rettgeri*, 5 of *Providencia alcalifaciens*, and 2 of *Providencia stuartii*. In addition to the 507 strains mentioned above, we tested 180 lactose-fermenting (in 18 to 24 h) strains of *Enterobactoriaceae*, including 108 *E. coli* strains, 51 *Klebsiella pneumoniae* strains, and 17 *C. freundii* strains. All of the strains were isolated in our laboratory from primary plating media in fecal cultures (MacConkey, xylose-lysine-deoxycholate, and Hektoen agars), and after being isolated again on MacConkey agar, the strains were identified by the standard criteria of Edwards and Ewing (4). *Yersinia* strains were excluded from the study because they are seldom isolated in our laboratory from primary plates (incubated 18 to 24 h at 35°C) in fecal cultures (isolation rate of 2×10^{-4} per plate). Similar data have been found in other laboratories in our area (14).

Inoculation and reactions in standard media, LIA, and the medium UMI. The standard media and the KIA-LIA combination were inoculated, incubated, and read according to the aforementioned criteria (4).

The UMI medium was inoculated by stabbing to the bottom of the tube with a straight wire. When the UMI medium was used in conjunction with KIA, KIA was inoculated in the usual way, and the UMI tube was inoculated subsequently, without going back to the colony (4). The UMI medium and all other media were incubated for 18 to 24 h at 35°C.

Urease activity was observed by a change of color to red, motility was observed by growth extending from the line of inoculation or diffuse turbidity of the medium, and indole production was observed by a pink to red reaction after the addition of 3 to 4 drops of Kovacs' reagent. The red color of phenol red in alkaline pH did not interfere because of the acidity of Kovacs' reagent.

Evaluation of the UMI medium. The 687 strains tested were simultaneously inoculated in UMI medium and standard media, and results were read after incubation.

Evaluation of the KIA-UMI and KIA-LIA combinations. The 507 non-lactose-fermenting strains tested were inoculated, as mentioned above, simultaneously in KIA-LIA and KIA-UMI. Each combination of media was inoculated from one colony from the secondary isolation on MacConkey agar.

Any strain with the following reaction pattern was considered as a possible *Salmonella* strain: (i) lysine decarboxylase positive and lysine deaminase negative on KIA-LIA; (ii) urease negative and indole negative on KIA-UMI. H_2S-negative or nonmotile strains were considered as possible *Salmonella* strains.

Any strain with the following reaction pattern was considered as a possible *Shigella* strain: (i) lysine decarboxylase negative, lysine deaminase negative, and H_2S negative on KIA-LIA; (ii) urease negative, motility negative, H_2S negative, indole negative, and non-gas producing or urease negative, motility negative, H_2S negative, indole positive, and gas producing on KIA-UMI. The indole-positive and non-gas–producing strains were not considered as possible *Shigella* strains.

Statistical evaluation. The significance of differences between standard media and UMI medium were evaluated by the binomial test (12). The relationship between reactions yielded by standard media and UMI medium was calculated by the chi-square (χ^2) technique with Yates' correction for continuity (12). The strength of the observed relationships was defined by the tetrachoric correlation coefficient (ϕ) (15). A P value of ≤ 0.01 was considered to be significant.

RESULTS

Evaluation of the UMI medium. The reactions of the 687 strains of *Enterobacteriaceae* tested on the UMI medium and standard media are shown in Table 1. All the reactions were clear and easy to read, although some indole-positive reactions (in nonmotile strains) were weaker in the UMI medium than in the standard test indole medium. Similar results have been published for the motility-indole-ornithine medium and the motility-indole-lysine medium (3, 11).

In the detection of urease activity, results with the UMI medium and the standard medium were identical for 665 strains (96.8% agreement; $\chi^2 = 599.2$; $P < 0.001$) and different for 22 strains. The UMI medium was less sensitive than the standard test medium in the detection of urease

TABLE 1. *Comparison of the number of strains of Enterobacteriaceae with positive (+) and negative (−) reactions on the standard medium (SM) and the UMI medium*

Test	No. of strains with the following reactions				% Agreement	ϕ	$P_{difference}$
	SM +, UMI +	SM +, UMI −	SM −, UMI +	SM −, UMI −			
Urease	334	18[a]	4[b]	331	96.8	0.93	0.0019
Motility	562	12[c]	4[d]	109	97.7	0.91	0.038
Indole	250	2[e]	1[f]	434	99.6	0.99	

[a] Of the 18 strains, 10 were *K. pneumoniae*, 6 were *S. marcescens*, and 2 were *C. freundii*.
[b] Of the 4 strains, 2 were *K. pneumoniae*, and 2 were *S. marcescens*.
[c] Of the 12 strains, 9 were *E. coli*, 2 were *P. mirabilis*, and 1 was *P. alcalifaciens*.
[d] *E. coli*.
[e] *K. pneumoniae*.
[f] *P. morganii*.

activity ($P_{difference}$ [P_{diff}] < 0.001). In detecting motility, the UMI medium performed as well as the standard test medium (99.7% agreement; $\chi^2 = 573$; $P < 0.001$), and the number of different reactions was not significant ($P_{diff} > 0.01$). In the detection of indole production, both the UMI and standard test media performed almost identically (99.6% agreement; $\chi^2 = 666.9$; $P < 0.001$).

Evaluation of the KIA-LIA and KIA-UMI combinations for the recognition of *Salmonella* species. The efficacy of KIA-LIA and KIA-UMI combinations for the presumptive recognition of *Salmonella* species is shown in Table 2. The KIA-UMI combination was more specific than the KIA-LIA combination in selecting reaction patterns compatible with *Salmonella* species ($P_{diff} < 0.001$).

Although there were many non-*Salmonella* strains with reaction patterns compatible with *Salmonella* species on KIA-LIA and KIA-UMI combinations, this was due primarily to the fact that H_2S-negative, nonmotile, or non-gas-producing (in KIA) strains were considered as possible *Salmonella* strains. However, this difficulty is more theoretical than real because most of these strains were β-galactosidase positive (*Enterobacter*, *Serratia*, or *Citrobacter*) or needed further evaluation as they had *Shigella* reactions.

Evaluation of the KIA-LIA and KIA-UMI combinations for the recognition of *Shigella* species. The efficacy of KIA-LIA and KIA-UMI combinations for the presumptive recognition of *Shigella* species is shown in Table 3. The KIA-UMI combination was superior to the KIA-LIA combination in its ability to differentiate and recognize *Shigella* species ($P_{diff} < 0.001$). With the KIA-LIA combination of media, further evaluation to exclude *Shigella* species was necessary in 51 of the 507 strains tested (10.1%), whereas, on the other hand, all of the strains with a *Shigella* reaction pattern in KIA-UMI were actually *Shigella* strains.

TABLE 2. *Comparison of the KIA-LIA and KIA-UMI combinations of media with non-lactose-fermenting (18 to 24 h) strains of Enterobacteriaceae for the recognition of Salmonella species*[a]

Comparison	No. of strains	% of total tested
Not *Salmonella* spp. on KIA-LIA and KIA-UMI	294	58.0
Possible *Salmonella* spp. on KIA-UMI but not on KIA-LIA	7[b]	1.4
Possible *Salmonella* spp. on KIA-LIA but not on KIA-UMI	63[c]	12.4
Possible *Salmonella* spp. on KIA-LIA and KIA-UMI	143[d]	28.2

[a] $\chi^2 = 261$; $P < 0.001$; $\phi = 0.72$; $P_{diff} = 1.14 \times 10^{-12}$.
[b] Of the 7 strains, 5 were *C. freundii*, and 2 were *E. aerogenes*.
[c] Of the 63 strains, 60 were *E. coli*, and 3 were *P. morganii*.
[d] Of the 143 strains, 4 were *E. aerogenes*, 3 were *E. cloacae*, 40 were *S. marcescens*, 5 were *S. liquefaciens*, 40 were *S. enteritidis*, 8 were *S. typhi*, 23 were *S. flexneri*, 18 were *S. sonnei*, and 2 were *S. boydii*.

TABLE 3. *Comparison of the KIA-LIA and KIA-UMI combinations of media with non-lactose-fermenting (18 to 24 h) strains of Enterobacteriaceae for the recognition of Shigella species*[a]

Comparison	No. of strains	% of total tested
Not *Shigella* spp. on KIA-LIA and KIA-UMI	413	81.5
Possible *Shigella* spp. on KIA-UMI but not on KIA-LIA	0	0.0
Possible *Shigella* spp. on KIA-LIA but not on KIA-UMI	51[b]	10.1
Possible *Shigella* spp. on KIA-LIA and KIA-UMI	43[c]	8.5

[a] $\chi^2 = 200.6$; $P < 0.001$; $\phi = 0.63$; $P_{diff} = 4.4 \times 10^{-16}$.
[b] Of the 51 strains, 24 were *E. coli*, 24 were *P. morganii*, 2 were *E. aerogenes*, and 1 was *S. marcescens*.
[c] Of the 43 strains, 23 were *S. flexneri*, 18 were *S. sonnei*, and 2 were *S. boydii*.

DISCUSSION

The importance of defecting urease activity in the rapid differentiation of enteric pathogens from the suspicious colonies selected from primary plating media in fecal cultures is well recognized (1, 4, 6, 7, 9, 11, 13). When working with one colony, it is usually only possible to inoculate two tubes of differential media because of the high risk of contamination involved in going back to the original colony (4). The problem of the primary recognition and differentiation of *Salmonella* and *Shigella* species (and other enteropathogens) is then focused on the use, as a whole, of two differential media providing a maximum of useful information when they are inoculated together from one colony.

The use of KIA (or triple sugar iron agar) in this way is almost universal, and its performance is supported by decades of usage. Several media have been devised for use in conjunction with KIA (or triple sugar iron agar), and perhaps the most widely employed is LIA. Recently, however, a new motility-indole-lysine medium (7, 11) that enables the detection of motility, indole production, and lysine decarboxylase production in one tube has been put forward for this purpose. Nevertheless, none of these media detect urease activity, which provides easier detection of *Proteus*, one of the genera more frequently picked from primary plating media in stool cultures. Lysine decarboxylase activity detection is not possible in some strains of *E. coli* in LIA in

18 to 24 h (4, 16) so that the reactions of these *E. coli* strains in LIA are the same as those of *Shigella* strains. Furthermore, neither LIA nor motility-indole-lysine medium is able to detect lysine deaminase activity in some *P. morganii* strains (4, 11, 16).

Therefore, we have designed and evaluated a medium (UMI) which enables the simultaneous determination of urease activity, motility, and indole production in one tube. This medium, when used together with KIA (KIA-UMI combination) is superior to the combination of KIA and LIA (KIA-LIA) for the presumptive recognition and differentiation of *Salmonella* and *Shigella* species in fecal cultures.

The UMI medium performs as well as the standard test media in the detection of motility and indole production in *Enterobacteriaceae*, and although it is less sensitive in the detection of urease activity, above all in *Klebsiella*, there is no problem when the UMI medium is used in fecal cultures because *Klebsiella* is not selected from the plating media.

Although *Yersinia* was excluded from this study, the KIA-UMI combination enables the presumptive recognition of this genus. When the KIA-UMI combination is used, *Yersinia* should be suspected in strains that in 18 to 24 h do not ferment lactose or produce either gas or H_2S in KIA, are not motile, and give a positive urease reaction, usually strong (18 to 24 h at 35°C), in UMI medium.

Results of this study show that the UMI medium, when used in conjunction with KIA (KIA-UMI), is more effective than the KIA-LIA combination in *Salmonella* differentiation because it enabled us to disregard *E. coli* strains that did not ferment lactose in 18 to 24 h and *P. morganii* strains that produced a negative lysine deaminase reaction (in LIA) and were not differentiated from *Salmonella* species by KIA-LIA. It is also important to point out that all the strains with reaction patterns compatible with *Salmonella* species on KIA-UMI were β-galactosidase-positive *Shigella* strains (with typical reactions) or actually *Salmonella* strains.

In the recognition and differentiation of *Shigella* species the KIA-UMI combination is still more efficient because all the strains with reactions compatible with *Shigella* species were actually *Shigella* strains, enabling us to discard a number of strains in which further testing would have been necessary had we used the KIA-LIA combination.

Results from this work and from our work with the UMI medium for 3 years in our laboratory indicate that the KIA-UMI combination enables the presumptive identification of *Shigella* species (which should be confirmed by agglutination with specific antisera) and *Salmonella* species (which should be confirmed with a rapid β-galactosidase test and agglutination with *Salmonella* antisera). Logically, all these presumptive identifications have to be confirmed by complete biochemical tests and serology.

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