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Modification of the Methodology of Stool Culture for *Salmonella* Detection

J. RUIZ,[1]* M. A. SEMPERE,[1] M. C. VARELA,[1] AND J. GOMEZ[2]

Sección de Microbiologia[1] and Unidad de Infecciosas,[2] Hospital Virgen de la Arrixaca, El Palmar, 30120 Murcia, Spain

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A total of 4,284 H_2S-positive colonies isolated on salmonella-shigella agar and 4,350 isolated on Hektoen agar were flooded with 5 μl of a reagent (MUCAP test; Biolife Italiana S.r.l., Milan, Italy) and observed after 3 to 5 min for the development of fluorescence produced in the presence of the C8 esterase enzyme. All of the 794 colonies isolated on salmonella-shigella agar and the 752 isolated on Hektoen agar and identified as positive for *Salmonella* spp. with conventional biochemical tests were found positive with the MUCAP test (the sensitivity was 100% and the negative predictive value was 100% for both media). Moreover, only six isolates identified by conventional biochemical tests as *Proteus vulgaris* were MUCAP test positive (the specificity was 99.8% and the positive predictive value was 99.2% for both media). On the basis of these results, we propose the use of the MUCAP test as a method for the screening of H_2S-positive colonies and only subculturing on Kliger agar of those colonies which are MUCAP test positive. The MUCAP test is a rapid method for the presumptive detection of *Salmonella* spp. and reduces the work and material involved in testing.

Gastrointestinal infections are still a worldwide public health problem, being among the primary causes of medical consultation (3, 8). Among the bacteria responsible for these syndromes, *Salmonella* spp. are one of the most common causes of bacterial diarrheal disease (7). The processing of a stool culture in the search for *Salmonella* spp. and other enteric pathogens by means of the usual method is slow and cumbersome. Accordingly, in this study we tried to reduce both the time and the effort needed for the diagnosis of *Salmonella* spp. by using a fluorescent reagent (MUCAP test; Biolife Italiana S.r.l., Milan, Italy) on H_2S-positive colonies isolated on solid media.

The MUCAP test reagent detects the C8 esterase enzyme present in *Salmonella* spp. and other bacteria. The enzyme acts on a substrate formed by an ester of 8 atoms of carbon conjugated with 4-methylumbelliferone, liberating this last compound, which is strongly fluorescent at 365 nm. The reagent is sterile and has no influence on the viability of the colonies to which it is added. The cost of each 5 μl is approximately $0.09.

The routine technique which we followed for the isolation of *Salmonella* spp. is the classical method of subculturing H_2S-positive or lactose-negative colonies isolated on media for enteric pathogens on Kliger agar. Depending on the reactions produced, we continued with biochemical tests using API 20E galleries (Biomerieux, Montelieu Vercieu, France) and antisera (Difco, Detroit, Mich.) for serogroup identification. Our study concentrated only on those H_2S-producing colonies which grew on salmonella-shigella (SS) agar or Hektoen agar. These colonies were flooded with 5 μl of MUCAP test reagent and observed for the development of fluorescence after 3 to 5 min at 365 nm. The absence of spontaneous fluorescence was checked before the reagent was added. After the reagent was added, the colonies were subcultured on Kliger agar for routine identification.

A total of 8,634 colonies isolated over a period of 2 years from 9,628 stool samples were studied. In 1,725 (17.9%) of the 9,628 stool cultures which were processed, there was growth of at least one enteric pathogen: 819 *Salmonella* spp. (46.9%), 772 *Campylobacter* spp. (44.7%), 71 *Shigella* spp. (4.1%), and 72 *Yersinia* spp. (4.2%) isolates.

Meanwhile, 400 strains of *Salmonella* spp. from our collection (160 serogroup D, 140 serogroup B, 90 serogroup C, and 10 unclassified) and 200 from the Centro Nacional de Microbiología, Virología, e Inmunología Sanitarias (Majadahonda, Madrid) (CNMVIS) (128 serogroup D, 38 serogroup B, 24 serogroup C_1, 6 serogroup E_1, 3 serogroup C_2, and 1 serogroup A) were plated on SS and Hektoen agars to test for fluorescence and/or production of H_2S. Also, MUCAP test reagent was used on 160 other strains of H_2S-positive bacteria (31 *Citrobacter freundii*, 69 *Proteus mirabilis*, and 60 *Proteus vulgaris* strains) to test for fluorescence.

A total of 4,284 H_2S-positive colonies were tested on SS agar, and 4,350 were tested on Hektoen agar. All of the colonies which produced fluorescence were identified as *Salmonella* spp., except for six *P. vulgaris* colonies isolated on both media (Table 1). The number of *Salmonella* spp. isolated on Hektoen agar was slightly lower than that isolated on SS agar (752 versus 794), although the false-positives were the same. The specificity, sensitivity, and positive and negative predictive values inferred from these results were 99.8, 100, 99.2, and 100%, respectively.

The 400 strains from our collection and the 200 from CNMVIS were found to be positive on both media, producing fluorescence and H_2S. On the other hand, none of the 160 H_2S-positive non-*Salmonella* strains used as a control were MUCAP test positive.

Our results coincide with those of Diaz et al. (1a), who obtained 149 positive results from 149 isolates of *Salmonella* spp. tested with the MUCAP test. There was also the added advantage that the test identified eight isolates of *Salmonella* spp. which were not detected by the standard method. Also, Aguirre et al. (1) identified 79 isolates of MUCAP test-positive *Salmonella* spp. from 83 isolates, although they later admitted that 3 of the remaining 4 which did not produce a positive reaction were mixed with other enteric flora and a later subculture on SS agar confirmed their

* Corresponding author.

TABLE 1. Results obtained with the MUCAP test on SS agar and Hektoen agar for H_2S-positive colonies

Organisms	No. of isolates detected by the MUCAP test on the following agar:			
	Hektoen		SS	
	Positive	Negative	Positive	Negative
Salmonella spp.	752	0	794	0
P. vulgaris	6	612	6	634
Other	0	2,980	0	2,850
Total	758	3,592	800	3,484

positivity. In our study, all of the colonies which were found positive for *Salmonella* spp. with the biochemical and serological test media were also found positive by the MUCAP test, including the 600 collection strains. On the other hand, only 5 of the other H_2S-positive strains (*P. vulgaris*) were MUCAP test positive, while the 160 control strains were negative.

The MUCAP test can also be applied to lactose- and H_2S-negative colonies on SS agar or Hektoen agar, although Kliger agar slants would have to be used if other enteric pathogens, such as *Shigella* or *Yersinia* spp., etc., were to be investigated. The MUCAP test applied in this way is also a rapid method for the presumptive diagnosis of *Salmonella* spp. when it is used in conjunction with the oxidase test (6). Although other rapid methods have been described (2, 4, 5), they are not as fast and economical as this one.

In conclusion, the MUCAP test is an easy and rapid method which allows the presumptive detection of *Salmonella* spp. on solid media in less than 5 min and also reduces the time and effort involved, since it is not necessary to subculture on Kliger agar H_2S-positive colonies that are MUCAP test negative.

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