JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1988, p. 2501–2504
0095-1137/88/122501-04$02.00/0

Vol. 26, No. 12

Evaluation of a Latex Agglutination Test for the Detection of *Salmonella* and *Shigella* spp. by Using Broth Enrichment

JENNIFER METZLER AND IRVING NACHAMKIN*

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4283

Received 23 May 1988/Accepted 7 September 1988

We evaluated the Bactigen *Salmonella-Shigella* Latex Agglutination Slide Test (Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) for detection of *Salmonella* and *Shigella* spp. in enrichment broth cultures (gram-negative broth incubated for 24 h) as part of the routine testing of stool samples. A total of 1,128 stool samples were screened by using this test. Of 29 samples culture positive for *Salmonella* spp., 25 were positive with the *Salmonella* test (sensitivity, 86.2%; specificity, 96.2%). Of four stool samples culture positive for *Shigella* spp., two were detected with the *Shigella* latex reagent. Overall, the *Shigella* test had a specificity of 99.2%. Testing of enrichment broth cultures after 24 h of incubation was more sensitive than was testing after 6 h of incubation. When used for direct culture identification, both reagents had a specificity of greater than 98.0%. We conclude that the *Salmonella* test may be useful as an enrichment broth screening test to detect *Salmonella* spp.; however, we cannot make any conclusions about the *Shigella* test because of the low number of culture-positive specimens in this study.

Traditionally, the detection of salmonellae and shigellae in stool specimens has relied upon the use of a variety of plate media and biochemical tests. Most clinical microbiology laboratories use a combination of nonselective, selective, and differential plate media and enrichment broth for stool cultures (4, 6). Suspect colonies from the various media are then screened to identify salmonellae and shigellae by using biochemical tests such as the triple sugar iron, lysine-iron, and Christiansen urea agar slants. If necessary, the organisms are identified through further biochemical testing. If the results indicate the presence of salmonellae or shigellae, serological typing is then performed (4).

Considering the low prevalence of disease, the time involved for performing cultures, and the cost, a rapid, cost-effective method for the detection of salmonellae and shigellae would be advantageous. By using a rapid method, one might be able to decrease the amounts of media and time needed to process negative stools.

We evaluated a latex agglutination test (Bactigen Salmonella-Shigella Latex Agglutination Slide Test [BSST]; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) as a screening test for detecting *Salmonella* and *Shigella* spp. in enrichment broth cultures after 18 to 24 h of incubation. We also determined the ability of the BSST to accurately identify suspect colonies from plate media.

MATERIALS AND METHODS

In the first part of the study, we prospectively tested 1,128 enrichment broth (gram-negative broth [GN]) cultures (Remel, Lenexa, Kans., and BBL Microbiology Systems, Cockeysville, Md.) with BSST and compared the results with those obtained by culture isolation. All samples were obtained from patients with diarrheal illnesses, were not selected for any particular characteristics, and were processed as soon as they were received in the laboratory. A total of 153 samples were obtained from the microbiology laboratory at The Children's Hospital of Philadelphia, and 975 were obtained from the clinical microbiology laboratory at the Hospital of the University of Pennsylvania. At The Children's Hospital of Philadelphia, stool samples or rectal swabs were plated on 5% sheep blood (BAP), MacConkey, and xylose lysine deoxycholate (XLD) agars, and a sample was also inoculated onto GN enrichment broth. Subcultures of enrichment broth culture were made to MacConkey and hecktoen enteric (HE) agar. At the Hospital of the University of Pennsylvania, stool samples were plated on BAP, MacConkey, and HE agars as well as in a GN enrichment broth culture. Subcultures from GN were plated onto MacConkey and XLD agar.

In order to study the effect of enrichment broth culture incubation time on the sensitivity of the BSST test, 276 enrichment broth cultures were tested after 6 to 8 h and 18 to 24 h of incubation. An additional 852 enrichment broth cultures were tested only after 18 to 24 h of incubation.

Testing was performed as recommended by the manufacturer. The BSST kit contains three latex reagents: one *Salmonella* latex and two *Shigella* latex reagents coated with specific antibodies (rabbit polyclonal). One drop of each of the three latex reagents was mixed with 50 μl of enrichment broth on a slide provided with the kit. The slide was rotated for 10 min at 100 to 160 rpm and then observed for the presence of agglutination. A reaction which was grainy or +/− was read as negative, and positive reactions were noted as 1+, 2+, 3+, or 4+ reactions, depending on the amount of agglutination present. A specimen that reacted with more than one latex reagent was considered to exhibit a nonspecific reaction.

In the second part of the study, we tested 70 enteric organisms from 15 genera from plate media with BSST reagents. These isolates had been frozen in brain heart infusion broth containing 15% glycerol and were streaked out on BAP, XLD, and HE agars, where appropriate. Suspensions of these isolates were prepared in 1.5 ml of a buffer supplied by Wampole Laboratories. Two McFarland turbidity standards were tested. Standards of 0.5 (low concentration) and 4.0 (high concentration) were used to determine whether agglutination reactions varied depending on

* Corresponding author.

TABLE 1. Detection of *Salmonella* and *Shigella* spp. in enrichment broth cultures with BSST after 18 to 24 h of incubation

BSST result	No. of culture results			
	Salmonella		Shigella	
	+	−	+	−
+	25	41	2	9
−	4	1,046	1	1,102
Nonspecific	0	12	1	13

the concentration of organisms present. Testing was performed as described above.

RESULTS

Detection after 18 to 24 h. After inoculation of GN enrichment broth with patient material, the enrichment broth was incubated for 18 to 24 h and tested with the BSST test. The results of testing after 18 to 24 h of incubation are shown in Table 1. Of 29 specimens containing salmonellae, 25 were detected by the *Salmonella* latex. Of the *Salmonella* isolates detected, 13 were serogroup D, 10 were serogroup B, 1 was serogroup O, and 1 was nongroupable. Four isolates of salmonellae were not detected by the BSST screening test. Two isolates belonged to group C2, one belonged to group B, and one belonged to group G. For salmonellae the sensitivity, specificity, and positive and negative predictive values were 86.2, 96.2, 37.9, and 99.6%, respectively.

Of four specimens containing shigellae (*Shigella soneii*), two gave a positive *Shigella* latex result. One specimen exhibited a nonspecific reaction, and one exhibited a negative reaction.

In order to determine the effect of incubation time on positivity rate, we tested 276 enrichment broth cultures after 6 to 8 h and 18 to 24 h of incubation (Table 2). After 6 to 8 h of incubation, the *Salmonella* reagent detected three of eight culture-positive samples (37.5% sensitivity). After 18 to 24 h of incubation, six of the eight culture-positive samples were detected (75.0% sensitivity). Only one sample was culture positive for shigellae in this experiment; however, the BSST did not detect it after 6 to 8 h or 18 to 24 h of incubation. The specificity of the *Shigella* latex was 99.3% for the test after 6 to 8 h and 99.6% for the test after 18 to 24 h.

TABLE 2. Detection of *Salmonella* and *Shigella* spp. in enrichment broth cultures with BSST after incubation for 6 to 8 or 8 to 24 h

Incubation time and culture type	No. with BSST test result of:			Sensitivity (%)	Specificity (%)
	+	−	Nonspecific		
6 to 8 h					
Salmonellae	3	5	0	37.5	98.5
Nonsalmonellae	4	261	3		
Shigellae	0	1	0	ND[a]	99.3
Nonshigellae	2	270	3		
18 to 24 h					
Salmonellae	6	2	0	75.0	96.6
Nonsalmonellae	9	255	4		
Shigellae	0	1	0	ND	99.6
Nonshigellae	1	270	4		

[a] ND, Not determined.

Direct testing of isolates. Clinical isolates of *Salmonella* and *Shigella* spp. and a variety of other bacteria were plated on BAP, XLD, and HE agar and tested with the BSST (Table 3). Both the *Salmonella* and *Shigella* latex reagents exhibited cross-reactions with *Klebsiella oxytoca* (n = 1), *Citrobacter diversus* (n = 1), and a non-lactose-fermenting *Escherichia coli* isolate (n = 1). These cross-reactions did not appear to be inoculum dependent, since the reactions were detected at both low and high inocula. There were nonspecific reactions (i.e., reactions with both *Shigella* and *Salmonella* latex), which occurred mainly with *Staphylococcus aureus* (n = 4). One isolate each of *K. oxytoca* and *Staphylococcus aureus* exhibited a nonspecific reaction at the high inoculum tested but was negative at the low inoculum.

Testing of *Salmonella* and *Shigella* isolates grown on different media varied slightly and was medium dependent. All nine *Salmonella* isolates reacted with the *Salmonella* latex when tested after being grown on HE agar, but one did not react with the latex when tested after being grown on blood agar or XLD agar. Similarly, one to two isolates of *Shigella* spp. did not react with the *Shigella* latex after being grown on blood agar or XLD agar, but all reacted when tested after being grown on HE agar.

DISCUSSION

Most laboratories use an enrichment broth to supplement primary plating media for optimizing the isolation of both *Salmonella* and *Shigella* spp. from fecal specimens. After the enrichment broth is incubated for up to 24 h, it is subcultured to selective media with subsequent screening and identification of colonies suspected of being one of these pathogens. In most laboratories, the percentage of stool cultures yielding one of these pathogens is low. At our institution, for example, approximately 2% of cultures yield *Salmonella* spp., whereas less than 1% yield *Shigella* spp. Because of the low prevalence of these diseases, the vast majority of the cost related to workup of enrichment broths is a result of negative rather than positive cultures.

There have been several types of tests for samonellae or shigellae or both which have become available in past years. However, there are disadvantages to many of them. For example, a 4-h multitest system, Micro-ID (General Diagnostics, Div. Warner-Lambert Co., Morris Plains, N.J.), was evaluated for the identification of colonies suspected of being salmonellae or shigellae (1). Although this test appears to have good performance, over one-third of cultures could not be tested because of inadequate growth for Micro-ID evaluation. Another test which screens colonies for salmonellae, shigellae, and yersiniae as an alternative to screening with biochemical tube media is the Rapid SST strip (DMS Laboratories, Inc.) (3). This test has the disadvantage of resulting in many false-positives (34%) compared with conventional screening methods (23%), and in our laboratory this made the screening of suspicious colonies by this method more costly than screening with biochemical tube media (unpublished data). Greene et al. also found that the Rapid SST strip was not highly reliable for screening colonies (2).

In our study, the BSST had good sensitivity, very good specificity, and negative predictive value when testing enrichment broth cultures for salmonellae. Because of the small number of *Shigella* isolates, we could not evaluate the sensitivity of the *Shigella* latex. However, the specificity was extremely good. As our results indicate and as was

TABLE 3. Colony identification from different growth media with *Salmonella* and *Shigella* latex reagents

Organism	No. tested	No. positive with *Salmonella* latex after growth on:			No. positive with *Shigella* latex after growth on:		
		BAP	XLD	HE	BAP	XLD	HE
Salmonella group D	2	2	2	2	0	0	0
Salmonella group B	4	4	4	4	0	0	0
Salmonella group C2	3	2	2	3	0	0	0
Shigella dysenteriae	1	0	0	0	1	1	1
S. boydii	1	0	0	0	1	1	1
S. soneii	4	0	0	0	4	4	4
S. flexneri	3	0	0	0	2	1	0
Aeromonas hydrophila	4	0	0	0	0	0	0
Pleisiomonas shigelloides	2	0	0	0	0	0	0
Vibrio parahemolyticus	2	0	0	0	0	0	0
Escherichia coli lf[a]	4	0	0	0	0	0	0
E. coli nlf[b]	7	0	0	0	1	1	0
Yersinia enterocolitica	2	0	0	0	0	0	0
Proteus spp.	4	0	0	0	0	0	0
Citrobacter freundii	2	0	0	0	0	0	0
C. diversus	2	0	1	0	0	0	0
Klebsiella oxytoca	3	1 (1)[c]	0 (2)	0 (2)	1 (1)	0 (2)	0 (1)
Klebsiella spp.	3	0	0	0	0	0	0
Enterobacter spp.	4	0	0	0	0	0	0
Pseudomonas aeruginosa	5	0	0	0	0	0	0
Staphylococcus aureus	4	0 (4)	0	0	0 (4)	0	0
Enterococcus spp.	4	0	0	0	0	0	0
Sensitivity (%)		88.9	88.9	100	77.8	88.9	100
Specificity (%)		98.2	98.0	100	98.3	98.3	98.3

[a] Lactose fermenting.
[b] Non-lactose fermenting.
[c] Values in parentheses represent number of isolates exhibiting a nonspecific reaction.

recommended by the manufacturer, optimal sensitivity of the BSST occurs when the GN broths are incubated for 18 to 24 h. The positive predictive value of the *Salmonella* test was approximately 38% and indicates that BSST should not be used to make a preliminary diagnosis of *Salmonella* infection. There were numerous enrichment broth cultures that exhibited a positive *Salmonella* test but in which we could not recover *Salmonella* spp. by culturing. The reasons for these false-positive results are not entirely clear. Our data show that the BSST *Salmonella* reagent exhibits some cross-reactivity with some isolates of the *Enterobacteriaceae*, albeit at a high inoculum. Thus, the presence of cross-reacting bacteria is likely to cause a false-positive result. We did not test bacterial isolates from false-positive enrichment broth cultures to determine the degree of cross-reactivity, however. A number of nonspecific reactions (i.e., reactivity of more than one latex reagent with the specimen) were also noted. These reactions were probably due to the presence of staphylococci or other bacteria in the enrichment broths that were shown to exhibit nonspecific reactions in the direct colony-testing study. None of the samples that exhibited a nonspecific reaction were identified as *Salmonella* spp.; however, one was identified as a *Shigella* sp. In the current test format, all enrichment broth cultures that exhibit nonspecific reactions should be subcultured. Thus, the likelihood of missing a significant pathogen with a nonspecific test result is extremely small. In spite of these results, the vast majority of culture-negative samples gave negative results with the BSST. With culturing results as the standard, the overall agreement of test results was 95.9%.

The advantages of employing the BSST in clinical laboratories include the savings in time and media costs involved when eliminating the subculture of the enrichment broth when the BSST gives a negative result. The savings would occur not only from the removal of the subculture plate(s) but from the resulting decrease in the number of colonies biochemically screened. Presently, our laboratory employs three primary agar plates (BAP, MacConkey, and HE), an enrichment broth (GN), and an XLD agar plate for the broth subculture when culturing stool samples for salmonellae and shigellae. The total cost, including technologist time and media costs, is approximately $1,100.00 per 100 stool samples. If our laboratory were to screen enrichment broths with the BSST and subculture only the reactive samples, the cost would be about the same. However, approximatley 11 h per 100 specimens would be saved in processing and workup time. Many of the laboratories in our area subculture their enrichment broths to MacConkey and XLD plates. Using a positive and nonspecific reaction rate of 7.6% (as shown in our study) with the BSST, these laboratories could achieve a significant savings of $251.12 and 21 h per 100 stools, assuming they have similar costs to ours, if they were to screen with the BSST.

The BSST becomes more advantageous in the clinical laboratory if the number of primary plates used is decreased. Not only would this decrease the amount of technologist time needed in culturing stools, reading plates, and screening colonies, but plate and biochemical medium costs would be significantly decreased. It might seem reasonable to exclude MacConkey agar from the battery of primary plate media. In a comparative study of XLD and MacConkey agar for detecting salmonellae and shigellae directly from clinical specimens, 2 of 75 (2.6%) *Salmonella* isolates grew only on MacConkey agar (5). Only 1 of 16 (6.2%) *Shigella* isolates grew only on MacConkey agar. A total of 60 of 75 (80%) *Salmonella* isolates and 15 of 16 (93.8%) *Shigella* isolates

were detected by XLD agar. These results suggest that laboratories may be able to eliminate MacConkey agar from the routine stool culture with no significant decrease in the isolation of salmonellae and shigellae. Further studies are needed to address this issue before reducing the number of primary plates presently used for routine stool cultures.

The results obtained when testing colonies directly from plate media indicate that the BSST would be a good method for screening suspicious colonies. In using the BSST, screening time would be decreased, as the biochemical tube media must incubate for 18 to 24 h and the BSST can be performed in 15 min. However, we noted some cross-reactions with bacteria other than *Salmonella* or *Shigella* spp., and thus additional methods should be used to confirm a positive result at the present time. Further studies involving the direct testing of colonies might include the comparison of the specificity and sensitivity of the BSST with those of the biochemical screening methods presently used.

In conclusion, we have found that the BSST may be a useful aid in screening stool samples for salmonellae and shigellae. Furthermore, clinical microbiology laboratories may find it beneficial to evaluate their present protocols for a routine stool culture to determine where the BSST would save time and decrease costs.

ACKNOWLEDGMENTS

This study was supported by a grant from Wampole Laboratories, a division of Carter-Wallace, Inc.

We thank Carol Jones for excellent secretarial assistance and the Children's Hospital of Philadelphia microbiology laboratory for providing clinical samples.

LITERATURE CITED

1. **Gooch, M. W.** 1980. Evaluation of a multitest system for rapid identification of Salmonella and Shigella. Am. J. Clin. Pathol. **73:** 570–573.
2. **Greene, L. C., P. C. Applebaum, and J. A. Kellogg.** 1984. Evaluation of a two-hour method for screening pathogens from stool specimens. J. Clin. Microbiol. **20:**285–287.
3. **Hinrichs, J., A. Gautnier, M. Keating, and P. Murray.** 1985. Evaluation of a new screening system for enteric pathogens. Diagn. Microbiol. Infect. Dis. **3:**397–401.
4. **Kelly, M. T., D. J. Brenner, and J. J. Farmer.** 1985. Enterobacteriaceae, p. 264–265. *In* E. H. Lennette (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Rollender, W., O. Beckford, R. Beisky, and B. Kostroff.** 1969. Comparison of xylose lysine deoxycholate agar and MacConkey agar for the isolation of Salmonella and Shigella from clinical specimens. Am. J. Clin. Path. **51:**284–286.
6. **Washington, J. A.** 1985. Initial processing for cultures of specimens, p. 113–115. *In* J. A. Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York.