JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2009, p. 456–458 Vol. 47, No. 2
0095-1137/09/$08.00+0 doi:10.1128/JCM.01643-08
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Evaluation and Implementation of a Chromogenic Agar Medium for *Salmonella* Detection in Stool in Routine Laboratory Diagnostics[▽]

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Received 24 August 2008/Returned for modification 30 September 2008/Accepted 7 December 2008

We evaluated which chromogenic agar medium for *Salmonella* detection in stool would be most sensitive and specific in our culture protocol. The use of BBL CHROMagar *Salmonella* chromogenic medium combined with xylose-lysine-deoxycholate agar yielded a sensitivity of 100% and also reduced workload and costs.

Salmonella detection in stool using conventional media, such as *Salmonella*-*Shigella* agar (SS), is based on lactose fermentation and H_2S production. The number of false-positive results with these media necessitates time-consuming and expensive additional testing. Recently, many chromogenic media with sensitivities and specificities higher than those of conventional media have been developed for the isolation of *Salmonella* (1, 6, 7).

We evaluated which of three chromogenic media would be an appropriate substitute for the traditional agar media in our culture protocol by using (i) stock isolates and (ii) clinical stool samples. We compared the sensitivities, specificities, and costs of the old and new methods, while safeguarding *Shigella* detection.

First, we evaluated three chromogenic media with 53 stock isolates stored frozen at −85°C, including 34 *Salmonella* strains (Table 1). We tested BBL CHROMagar *Salmonella* medium (BBL; BD Diagnostics, Erembodegem-Aalst, Belgium), Oxoid *Salmonella* chromogenic medium (OX; Oxoid, Basingstoke, United Kingdom), and SM ID2 medium (SM; bioMérieux, Marcy l'Etoile, France). Specific chromogenic enzyme substrates make most *Salmonella* isolates appear with mauve (BBL), magenta (OX), or pale-pink to mauve (SM) colonies. Isolates other than *Salmonella* spp. have different colors or are inhibited. All media were supplied as ready-to-use agar plates. From overnight cultures on sheep blood agar, 10-μl suspensions equivalent to a 0.5 McFarland standard were plated onto BBL, OX, and SM by use of a three-streak dilution method. The media were read after 24 and 48 h of incubation at 35°C.

On BBL, all stock salmonellae produced mauve or blue-violet (*Salmonella enterica* subsp. *arizonae* and *S. enterica* subsp. *diarizonae*) colonies after 24 and 48 h of incubation. No strain was inhibited. On OX, after 24 h, *Salmonella enterica* serovar Paratyphi A had pale-pink colonies and was strongly inhibited. After 48 h, *Salmonella* serovar Paratyphi A was still inhibited but showed the correct magenta color. *S. enterica* subsp. *arizonae* and *S. enterica* subsp. *diarizonae* produced blue colonies similar to those of other *Enterobacteriaceae*. On SM, both lactose-positive *S. enterica* subsp. *arizonae* strains produced lilac to pale-blue colonies.

Isolates other than *Salmonella* spp. with *Salmonella*-like colonies on BBL after 24 h of incubation were *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. After 48 h, other organisms that gave false-positive results were *Acinetobacter baumannii*, *Citrobacter freundii*, *Shigella dysenteriae*, and *Candida tropicalis*. On OX, organisms that gave false-positive results after 24 h were *A. baumannii*, *A. hydrophila*, *P. aeruginosa*, *S. dysenteriae*, and *Candida albicans*. After 48 h, *Citrobacter koseri*, *Morganella morganii*, *Proteus mirabilis*, and *Shigella boydii* gave false-positive results as well. On SM, organisms that gave false-positive results after 24 h were *A. baumannii* and *P. aeruginosa*, and after 48 h *A. hydrophila*, *C. albicans*, and *C. tropicalis* also gave false-positive results. Overgrowth of coliforms other than *Salmonella* spp. was lowest on BBL. Table 2 shows the sensitivities and the specificities of the chromogenic media calculated after stock isolate testing. Based on these findings, we continued the study with BBL and SM.

Second, stool samples from general-practice patients with gastroenteritis submitted to our laboratory and to the Laboratory for Infectious Diseases (LvI), Groningen, The Netherlands, were cultured according to the current protocol. Approximately 50 μl of a stool suspension (1.5 g in 6 ml 0.85% NaCl) was plated onto SS, xylose-lysine-deoxycholate agar (XLD), and Hektoen enteric agar (Oxoid). Additionally, a gram-negative broth (Difco GN broth; BD) was inoculated and subcultured onto the same media after overnight incubation at 35°C. BBL and SM were inoculated together with the traditional media, directly and after enrichment. Direct cultures and subcultures were evaluated for colonies suspected of being *Salmonella* and for inhibition of normal fecal flora after 24 and 48 h of incubation at 35°C.

Suspect colony types, which tested oxidase negative, were biochemically screened using test media, including triple sugar iron agar, urea agar, and lysine decarboxylase medium. Presumptive *Salmonella* isolates were confirmed by using a Vitek 2 system (bioMérieux) and by seroagglutination.

For calculating the sensitivities and the specificities of the media, McNemar's test was used. We compared the levels of inhibition of normal flora by using the Wilcoxon signed rank test.

Of the 1,339 cultured stool samples, 32 (2.4%) were *Salmonella* positive on at least one medium, which is consistent with

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▽ Published ahead of print on 17 December 2008.

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TABLE 1. Stock isolates used for testing three chromogenic *Salmonella* agar media

Species	No. of strains	Details
Salmonella strains		
Serogroup I		
Serovar Enteritidis	5	Including one LDC[a]-negative strain
Serovar Typhimurium	5	Including ATCC 14028
Serovar Typhi	2	
Serovar Paratyphi A	1	ATCC 25923
Serovar Paratyphi B	3	
Others	8	
Serogroup III		
S. enterica subsp. *diarizonae*	1	
S. enterica subsp. *arizonae*	4	Including two lactose-positive strains
Serogroups II and IV	5	
Total	34	
Strains other than *Salmonella* spp.		
Acinetobacter baumannii	1	
Aeromonas hydrophila	1	
Citrobacter freundii	1	
Citrobacter koseri	1	
Enterobacter cloacae	1	ATCC 13047
Escherichia coli	1	ATCC 25922
Klebsiella pneumoniae	1	ATCC 13883
Morganella morganii	1	
Proteus mirabilis	1	
Pseudomonas aeruginosa	1	ATCC 27853
Shigella boydii	1	
Shigella dysenteriae	1	
Shigella flexneri	1	
Shigella sonnei	1	ATCC 25931
Yersinia enterocolitica	1	
Candida albicans	1	ATCC 2091
Candida tropicalis	1	
Enterococcus faecalis	1	ATCC 29212
Staphylococcus aureus	1	ATCC 25923
Total	19	

[a] LDC, lysine decarboxylase.

isolation rates from previous Dutch reports (3, 8). Table 3 shows the sensitivities and specificities of the tested media. The sensitivity of each medium was highest after reading the direct plate after 48 h and including enrichment subcultures. The results did not change after reading the subcultures after 48 h. Thirty-one *Salmonella* isolates were detected on XLD, and the one strain missed on XLD was found solely on BBL. XLD and BBL combined would therefore yield a sensitivity of 100%.

TABLE 2. Sensitivities and specificities of the tested media after stock isolate testing

Medium	Sensitivity (%)		Specificity (%)	
	24 h	48 h	24 h	48 h
BBL	100.0	100.0	84.2	68.4
OX	85.3	85.3	73.7	52.6
SM	94.1	94.1	89.5	78.9

TABLE 3. Sensitivities and specificities of the tested media calculated from culture results after 48 h and including enrichment

Medium	No. of *Salmonella* isolates	Sensitivity (%)	No. of false-positive strains	Specificity (%)
SS	23	71.9	277	78.8
XLD	31	96.9	261	80.0
HEA[a]	27	84.4	245	81.3
BBL	28	87.5	56	95.7
SM	25	78.1	51	96.1

[a] HEA, Hektoen enteric agar.

As expected, the traditional media yielded the most false-positive strains. Specificities of the chromogenic media were significantly higher ($P < 0.05$). Organisms that gave false-positive results with BBL and SM were *Escherichia coli*, *M. morganii*, *Hafnia alvei*, and *Pseudomonas* spp.

SM showed significantly more growth of normal fecal flora than BBL ($P < 0.001$).

Eigner et al. tested BBL with stool samples and found a sensitivity of 85% and a specificity of 99% after 48 h of incubation of direct plates (5). In two other studies, BBL and SM were compared using stock isolates and stool samples, respectively (2, 4). In both studies, after 48 h BBL had a lower sensitivity but a higher specificity than SM. Still, the sensitivity rates of BBL were 90 and 98.1%, respectively. Delorme et al. determined a lower selectivity for SM than for BBL (2), which is consistent with the larger amount of fecal overgrowth we saw on SM.

In our revised protocol, we plate stool samples directly and after enrichment in GN broth onto BBL and XLD, striving for a high sensitivity of *Salmonella* detection and optimal *Shigella* isolation. For the approximately 4,600 stool samples we process each year, we calculated that prices for inoculation, subcultures, screening, and confirmation using traditional media would be €24,150, €42, €5,015, and €6,181, respectively (total of €35,388), with the current protocol, versus €23,414, €104, €1,133, and €3,646 (total of €28,297), respectively, with the new protocol. Adding the saving of technician time, we estimate a total yearly cost reduction of 27% (€12,364).

We thank BD Diagnostics, bioMérieux, Oxoid, and the LvI for contributing to this study.

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