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Evaluation of the Wellcolex Colour Salmonella Test for Detection of *Salmonella* spp. in Enrichment Broths

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The Wellcolex Colour Salmonella Test was evaluated for detection of *Salmonella* spp. in enrichment broths of 1,010 stool samples. In 39 specimens, *Salmonella* spp. could be isolated from the selenite F broth (SF). Wellcolex agglutination indicative of the presence of *Salmonella* spp. was noted with the SF in 36 cases, 34 of which were in agreement with the subculture results. Therefore, relative to subculture, the sensitivity and specificity of the Wellcolex-selenite F procedure were 87 and 99%, respectively. Five false-negative results were noted. The gram-negative broth (GN) subculture revealed only 23 *Salmonella* spp. (59% sensitivity). The Wellcolex agglutination procedure applied to the GN indicated *Salmonella* spp. for 21 samples; its sensitivity was 70% and its specificity was 99% compared with GN subcultures. The Wellcolex agglutination procedure applied to the SF performed better than the agglutination of GN or direct plating procedures and detected 17 of the 39 *Salmonella* spp. at least 24 h earlier than did culture.

The detection of *Salmonella* spp. in stool specimens by traditional culture techniques is time-consuming and requires a large amount of media and a high level of technical skill. The delay of up to four days after sampling for definite identification of *Salmonella* spp. is long, especially if effective quinolone treatment is to be instituted (10, 14, 16). The many reports on *Salmonella* epidemics emphasize the need to improve conventional detection methods (3, 17, 20). Latex agglutination methods seem to facilitate the detection of salmonellae (1, 5, 9, 12, 13).

A new colored-latex test has been described recently (7). The reagent contains differently colored latex suspensions, each having been coated with a specific antibody before mixing. On the basis of this technology, Wellcome Diagnostics UK marketed the Wellcolex Colour Salmonella Test. With only two reagents, it allows the detection and identification of *Salmonella* groups A, B, C, D, and either E or G.

(These results were presented in part at the 91st General Meeting of the American Society for Microbiology [3a].)

This Wellcolex Test was evaluated on 1,010 routine stool samples from patients with diarrheal illness. These specimens were plated onto MacConkey agar (Oxoid, Basingstoke, United Kingdom) and Hektoen enteric agar (Diagnostics Pasteur, Paris, France); approximately 1 g was inoculated into both 10 ml of selenite F broth (BBL Microbiology Systems) and 10 ml of gram-negative broth (BBL Microbiology Systems). The primary plates and the two broths were incubated for 18 to 24 h at 35°C. We followed this work flow in a manner similar to that of many other microbiology laboratories; because of insufficient staff, many laboratories cannot perform the optimal subculture of selenite F broth, after 8 to 12 h of incubation, and of gram-negative broth, after 4 to 6 h of incubation (11).

The broths were subcultured onto Hektoen enteric agar and MacConkey agar. Suspect colonies on the primary or subculture plates were screened for *Salmonella* spp. by applying lysis by the 01 *Salmonella* bacteriophage (Diagnostics Pasteur, Paris, France), serological tests (Behring, Marburg, Germany), and biochemical tests (6, 8, 15). The same enrichment broths were used to carry out the Wellcolex Colour Salmonella Test, which consists of two latex reagents and three positive controls. Reagent 1 contains latex particles of three colors coated with rabbit antibodies specific for *Salmonella* group B (red), group C (blue), and group D1 (green). Reagent 2 also contains latex particles of three colors: red particles are coated with anti-Vi antigen antibodies, blue particles are coated with anti-group E and anti-group G antibodies, and green particles are coated with anti-group A antibodies. After being heated for 5 min in a boiling water bath, a 40-μl sample from the selenite F or gram-negative broth was transferred to each of two circles on a disposable card. One drop of latex reagent 1 was added to one circle, and latex reagent 2 was added to the other. The broth was mixed with the latex suspension, and the card was then placed on a rotator and run at 150 rpm for 2 min. A red, blue, or green macroscopic agglutination was interpreted as a positive reaction.

In 39 samples (4%), *Salmonella* spp. could be cultured from the selenite F broth; one belonged to serogroup A (*Salmonella paratyphi* A), five belonged to serogroup B (two *Salmonella typhimurium*, one each of *Salmonella brandenburg*, *Salmonella chester*, and *Salmonella wien*), four belonged to serogroup C, and 29 belonged to serogroup D (28 *Salmonella enteritidis* and one *Salmonella typhi*). On the primary plates, 22 strains (56%) were detected which were also isolated from the selenite F broth subcultures (Table 1).

The Wellcolex Colour Salmonella Test applied to selenite F broth indicated the presence of *Salmonella* spp. in 36 cases; 34 cases were in agreement with the results of selenite F broth subcultures. These Wellcolex results agreed with the results of subsequent serological identifications. The two nonmatched positive Wellcolex reactions were positive for *Salmonella* group E or G (Table 1). These results were clearly distinct from nonspecific agglutinations. In 3% of the samples, nonspecific agglutination, characterized by brownish discoloration with coarse clumps, was observed simultaneously with both Wellcolex reagents. Eight Vi antigen-positive reactions were interpreted as negative for *Salmonella* spp. according to the manufacturer's recommenda-

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TABLE 1. Comparison of primary plate cultures, selenite F broth, and gram-negative broth subcultures with Wellcolex Colour Salmonella Test for detection of *Salmonella* spp.

Detection method[a]	No. identified as *Salmonella* group:					Total no. detected
	A	B	C	D	E or G	
Primary plate		1	2	19		22
SF agglutination	1	4	4	25[b]	2	36
SF subculture	1	5	4	29[b]		39
GN agglutination		1	3	14	3	21
GN subculture		2	1	20		23

[a] SF, selenite F broth; GN, gram-negative broth.
[b] One *Salmonella typhi* was positive for group D and Vi.

tion. The *Salmonella typhi* reacted with both Vi antigen and serogroup D reagents. The Wellcolex Colour Salmonella Test did not detect four *Salmonella enteritidis* and one *Salmonella wien*. The sensitivity, specificity, and predictive value of a positive and a negative result for the Wellcolex agglutination of the selenite F broth were 87, 99, and 94 and 99%, respectively. This agglutination detected 17 of the 39 *Salmonella* spp. (44%) at least 24 h earlier than the conventional cultures.

Only 23 of the 39 *Salmonella* spp. grown from selenite F broth could be isolated from gram-negative broth (sensitivity, 59%) (Table 1). No additional *Salmonella* spp. could be grown from gram-negative broth, and 17 of the 23 *Salmonella* spp. were isolated from the primary plates.

Applied to the gram-negative broth, the Wellcolex Colour Salmonella Test indicated the presence of *Salmonella* spp. in 21 specimens, of which 16 could be cultured (Table 1). Thirteen other positive agglutinations were noted: three for the antigen E or G, two for serogroup C, and eight for the Vi antigen. The latter eight were not interpreted as *Salmonella* spp. Thus, comparison of these Wellcolex results to the results of gram-negative broth subcultures resulted in sensitivity, specificity, and predictive value of a positive and a negative for the Wellcolex Colour Salmonella Test of 70, 99, and 76 and 99%, respectively.

The superiority of broth enrichment over primary plate culture that we observed has also been reported by others (5, 19). The inadequate sensitivity of the gram-negative broth subculture (59%) compared with the sensitivity of the selenite F broth may be due to overgrowth of normal fecal microorganisms, because this broth was subcultured after 18 to 24 h, not after the optimal incubation time of 4 to 6 h. In this setting, which is the situation in many other laboratories, we would not recommend the gram-negative broth for the isolation of *Salmonella* spp. (4, 11).

In our routine clinical bacteriology laboratory, an improvement in the detection of *Salmonella* spp. in stool specimens could be achieved with the Wellcolex Colour Salmonella Test when it was used with selenite F broth. It was simple to use, and the positive results were easily interpretable. However, the green agglutination of the *Salmonella* group D antigen in reagent 1 was sometimes difficult to read. Because this group is common in our region, it may be worth changing the color assigned to group D. The five *Salmonella* spp. cultured from selenite F broth but not detected by the Wellcolex Colour Salmonella Test may not have reached sufficient numbers to allow detection by agglutination. If their growth could be enhanced, the sensitivity of the test might be increased. The two false-positive agglutinations for *Salmonella* group E or G could be due to the presence of lactose-positive *S. arizonae*, missed in our routine cultures. The eight reactions observed with the Vi antigen reagent without *Salmonella* spp. being detected in cultures were probably due to common Vi antigens found in certain *Citrobacter* spp. (18).

The poor sensitivity (70%) of the Wellcolex Colour Salmonella Test when used with the gram-negative broth is probably due to the small number of *Salmonella* spp. in this broth. The abundant growth of other enteric bacteria in this broth could have inhibited the growth of the *Salmonella* spp., which could also explain the relatively large number of false-positive reactions causing a low positive predictive value of 76%.

It is worth considering the Wellcolex Colour Salmonella Test for the detection of *Salmonella* spp. when used with the selenite F broth enrichment, because of its predictive value of a positive and a negative specimen. In this study, the agglutination of the selenite F broth detected more *Salmonella* spp. than the primary plates or gram-negative broth (subcultured after 18 to 24 h). In addition, these *Salmonella* spp. could be detected more rapidly. From this point of view, one primary plate for stool cultures would be sufficient; it can be chosen for the optimal isolation of *Shigella* spp. (4, 19). Economies are furthermore possible relative to the use of bacteriophage reagents, biochemical screening tests, serological confirmation tests, and personnel time. In other studies, this test has also been successfully used to screen lactose-negative colonies on primary plates or for serological grouping (2, 9). Selenite F broth subcultures should be maintained to confirm the Wellcolex Colour Salmonella Test result, to isolate and identify biochemically the *Salmonella* spp., and to detect the rare *Salmonella* groups not covered by the Wellcolex Colour Salmonella Test (<2%) (9). Furthermore, additional *Salmonella* spp. may be detected because of the higher sensitivity of the selenite F subculture over primary plating. The Wellcolex Colour Salmonella Test allows an early diagnosis of salmonellosis, which is important to relieve patients from diarrheal illness and to prevent epidemics caused by carriers, especially since quinolone treatment seems to be effective therapy (10, 14, 16).

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