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

PETER ROHNER,* SASI DHARAN, AND RAYMOND AUCKENTHALER
Central Bacteriological Laboratory, University Hospital Geneva, 24, Rue Michelli-du-Crest, 1211 Geneva 4, Switzerland

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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 in 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 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 (Diagnostica 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 (Diagnostica 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-

* Corresponding author.
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.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>No. identified as Salmonella group:</th>
<th>Total no. detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Primary plate</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>SF agglutination</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SF subculture</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>GN agglutination</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>GN subculture</td>
<td>2</td>
<td>1</td>
</tr>
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a SF, selenite F broth; GN, gram-negative broth.

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 gram-negative 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 and 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 detect 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).

REFERENCES
ratories will undertake this specialized testing. We would like to reemphasize that Yersinia strains can be immediately subcultured from primary plates to CR-MOX agar. Most pathogenic strains will be apparent after overnight incubation at 36°C because of the characteristic small red colonies they will produce.

We used the term pathogenic serotypes because it has been widely used in the literature to indicate strains that are potential enteric pathogens. Chiesa et al. encountered strains of Y. enterocolitica (usually from environmental sources) that agglutinated in 6 of the 11 sera used to define pathogenic serotypes: O3; O4,32; O8; O18; O20; and O21. However, these strains had other phenotypic properties indicating that they are not enteric pathogens. For example, they found 19 strains that agglutinated in O3 antiserum but were pyrazinamidase, salicin-esculin, and xylose positive. These strains would be classified as serotype O3, a pathogenic serotype, yet they would not be considered enteric pathogens. This conflicting nomenclature could easily be confusing, particularly to those not familiar with the subtleties of pathogenicity in the genus Yersinia. We agree with Chiesa et al., who used the term pathogenic phenotype, that the term pathogenic serotype can occasionally be very misleading. Perhaps it is time to replace it with a more precise term such as pathogenic phenotype, pathogenic bio-serotype, or pathogenic bio-serogroup.

J. J. Farmer III
G. P. Carter
I. K. Wachsmuth
Division of Bacterial and Mycotic Disease
National Center for Infectious Diseases
Centers for Disease Control and Prevention
Atlanta, Georgia 30333

V. L. Miller
S. Falkow
Department of Medical Microbiology
Sanford University
Sanford, California 94305

Wellcolex Colour Salmonella Test and Selenite-F Broth

I read with interest a recent publication entitled "Evaluation of the Wellcolex Colour Salmonella Test for Detection of Salmonella spp. in Enrichment Broths" (6). However, we would like to make a number of comments on the results of the Wellcolex Colour Salmonella Test in the above publication, as they are significantly different from our own experience with the test (5).

In the study by Rohner et al., the sensitivity of the Wellcolex test is lower, even with the Selenite-F broth, than those in other studies (1, 3, 5). A number of key factors were omitted in this study which could explain the lower performance of the Wellcolex Salmonella Test.

(i) Amount of inoculum. The manufacturer gives precise instructions regarding the amount of inoculum to be used for a specific volume of Selenite broth, as it is added at a critical stage for optimum recovery of Salmonellae. Seeding Selenite broth with too little or too much would lead to poor growth of salmonellae.

(ii) Emulsification. Emulsification of fecal specimens prior to inoculation is also recommended by the manufacturer, as this should liberate Salmonella spp. and allow maximum growth in this selective environment.

(iii) GN broth. The recommended incubation time for GN broth is 6 to 8 h (2). In the study by Rohner et al. (6), the incubation time was 18 to 24 h, which would indicate that the laboratory procedure did not use culture conditions for optimal recovery of Salmonella spp. This appears to be confirmed by the fact that the subcultures from the GN broth missed five Salmonella spp. which were isolated with the primary plates. There was therefore no point in evaluating the performance of the Wellcolex Colour Salmonella Test on this GN broth. Furthermore, the manufacturer recommends testing only on Selenite-F Broth.

(iv) Quality of the Selenite-F broth. Lastly, the quality of the Selenite-F broth is obviously critical in the recovery of Salmonella spp. The above study (6) only compared GN and Selenite-F broths, without comparing different Selenite broths. Our own evaluation (5) has shown quality differences between Selenite-F broths from two manufacturers.

By changing the methodology as described above, we found that the sensitivity of the Wellcolex Colour Salmonella Test was 99% on Selenite-F broth (5). It should also be noted that the salmonella incidence in our study was over 20%, compared with 4% in the study by Rohner et al.

We wish to publish this letter in reply to Dr. Rohner's publication, as we feel that the results of their study are incomplete and do not represent the true performance of the Wellcolex Colour Salmonella Test.

REFERENCES

Beatrix Orden
Ana Franco
Sección de Microbiología
Servicio de Análisis Clínicos
Ambulatorio "Arguelles"
Quintana 11
28008 Madrid
Spain
Authors' Reply

For the Wellcolex Colour Salmonella Test using Selenite-F broth, sensitivities ranging from 62 to 100% have been reported (1, 3, 4). Since these values depend on the quality of the reference culture method, the true sensitivity of the Wellcolex Colour Salmonella Test can be expected to be somewhere between the two. We achieved a sensitivity of 87% (5), which is higher than the sensitivities of 62.1% (4) and 83.1% (3) communicated in abstracts by B. Orden and coworkers. Our sensitivity results are therefore not significantly lower than those found in other studies, as stated in the letter by Orden and Franco. Since they have not cited or published the evaluation in which they achieved a sensitivity of 99%, we cannot comment on their results (3, 4).

We believe that no key factors were omitted in our study. First, our method of Selenite-F inoculation follows exactly the recommendations of the manufacturer, Becton Dickinson: ‘‘For feces and other solid materials, suspend 1 or 2 g of the specimen in the broth (approximately 10 to 15% by volume) and emulsify with an inoculating needle, if necessary.’’ This is mentioned in the Materials and Methods section of our paper (5). Second, as it is a basic procedure in stool cultures to emulsify the few solid fecal specimens, we did not mention this detail.

Third, the reasons for evaluating the Wellcolex Colour Salmonella Test with gram-negative broth incubated for 18 to 24 h at 35°C are explained in the third paragraph of our paper. The survey we cite (2) indicates that 23 of 26 laboratories use gram-negative broth for stool cultures and that only 5 of these perform subcultures after 4 to 6 h of incubation. Our study indicates to what extent an incubation of gram-negative broth for ≥18 h would miss Salmonella spp. in stools. Lastly, the aim of our study (5) was to evaluate the Wellcolex Colour Salmonella Test and not to compare the performance of Selenite-F from different sources. We may assume that Selenite-F brands with a high yield of Salmonella spp. in subcultures would achieve comparable results with the Wellcolex Colour Salmonella Test.

For hospitalized patients, from whom we receive specimens, the incidence of Salmonella spp. in feces is normally lower than that for ambulatory patients. Moreover, this prevalence can differ significantly from one country to another. In the context of analyzing stool specimens of a population with a low salmonella incidence, the specificity (99%) of the Wellcolex Colour Salmonella Test in our hands is remarkable.

REFERENCES


Peter Rohner
Sasi Dharan
Raymond Auckenthaler
Central Bacteriological Laboratory
University Hospital Geneva
24, rue Micheli-du-Crest
1211 Geneva 4
Switzerland