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Evaluation of Two Colored Latex Kits, the Wellcolex Colour Salmonella Test and the Wellcolex Colour Shigella Test, for Serological Grouping of *Salmonella* and *Shigella* Species

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Two colored latex kits (the Wellcolex Colour *Salmonella* Test [WCT-Salmonella] and the Wellcolex Colour *Shigella* Test [WCT-Shigella]; Division Diagnostics, Laboratoires Wellcome S.A., Paris, France), which allow identification of the most frequently encountered *Salmonella* serogroups and *Shigella* species, respectively, were evaluated. WCT-Salmonella and WCT-Shigella yielded sensitivities of 98.4 and 98%, respectively, and a specificity of 100% when they were tested on pure cultures received at a reference laboratory.

Serotypes of *Salmonella* are defined on the basis of their antigenic structure and occasionally on the basis of additional biochemical reactions (2). The Kauffmann-White scheme (5) currently lists 2,213 serotypes belonging to 67 somatic antigen groups. Determination of specific serotypes requires the availability of an extensive battery of antisera. Thus, most clinical laboratories report the serogroup (2) and send their isolates to a reference laboratory for further typing. Some invasive serotypes, namely, *Salmonella* serotype Typhi, *Salmonella* serotype Paratyphi A, and *Salmonella* serotype Choleraesuis, should be specifically identified because of their special clinical significance. A few biochemical characteristics allow identification of these invasive *Salmonella* serotypes, which are responsible for human enteric fever (2). Furthermore, the identification of the specific antigens by clinical laboratories (somatic antigen O:9 combined with Vi antigen for *Salmonella* serotype Typhi and somatic antigen O:2 for *Salmonella* serotype Paratyphi A) allows an accurate confirmation of invasive *Salmonella* for physicians. Other *Salmonella* serotypes of human origin are biochemically quite similar. When a *Shigella* species is suspected by a clinical laboratory, biochemical identification to the genus level is performed on an isolated lactose-negative colony and the species is serologically determined. The four species of *Shigella*, *Shigella dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D), can be distinguished by serological reactions. The isolate is often sent to a reference laboratory for complete identification.

Latex agglutination tests have been applied to the detection of a wide variety of antigens and antibodies (6, 7). The use a novel colored latex test for the detection of *Salmonella* serotypes was recently described (1). Two colored latex agglutination tests, the Wellcolex Colour *Salmonella* Test (WCT-Salmonella) and Wellcolex Colour *Shigella* Test (WCT-Shigella) (Division Diagnostics, Laboratoires Wellcome S.A., Paris, France) are now available. The manufacturers note that these tests can be performed on non-lactose-fermenting colonies growing in primary culture on selective media (for example, MacConkey agar and Hektoen enteric agar), in subculture from enrichment broth on these media, or in pure culture (for example, nutrient agar). Furthermore, WCT-Salmonella may also be performed directly on enrichment media (for example, Selenite-F broth). These tests are based on agglutination of antibody-coated colored latex particles in the presence of homologous antigens. Wellcome Laboratories claims that with only two reagents, WCT-Salmonella allows the determination of the most frequently encountered *Salmonella* serogroups (somatic O group A, B, C, D, E, and G and Vi antigens). It is also claimed that with only two reagents, WCT-Shigella allows the identification of *Shigella* isolates to the species level.

The purpose of this study was the evaluation of WCT-Salmonella and WCT-Shigella on pure cultures sent as presumptive *Salmonella* or *Shigella* isolates by clinical laboratories to the French National *Salmonella* and *Shigella* Center (CNS).

One hundred ninety-three isolates sent as presumptive *Salmonella* isolates were randomly chosen from a total of 2,258 isolates received at the CNS during a 2-month period (November and December 1989). One hundred sixty-three strains (84.5%) were of human origin (blood, stool, urine, or unknown human origin), 23 (11.9%) were of veterinary origin, and 7 (3.6%) were from miscellaneous sources. One hundred three isolates sent as presumptive *Shigella* strains during the period from June 1989 to February 1990 were examined. All *Shigella* strains were isolated from human stools. The strains were streaked on Drigalski's lactose-crystal violet agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) and checked for purity. At this stage, two tryptocasein soy agar slants (Sanofi Diagnostics Pasteur) were inoculated with lactose-negative colonies. After incubation, the bacteria grown on one slant were used at the reference laboratory for confirmation of identification and antigenic analysis, and the bacteria grown on the other slant were processed in blind fashion by another laboratory worker (without further studies) for the evaluation of the kits. Reference antigenic analysis was performed by slide agglutination with commercially available antisera (Sanofi Diagnostics Pasteur) or, if such antisera were not available, antisera prepared at the CNS.

WCT-Salmonella and WCT-Shigella. Kits for these tests were composed of two reagents, with each reagent containing colored latex particles (red, blue, and green latex particles in WCT-Salmonella reagents and blue and red particles

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TABLE 1. Interpretation of the colors of the observed aggregates in WCT-Salmonella

Reagent	Serogroup or antigen determined by indicated color of aggregates[a]		
	Green	Blue	Red
Latex 1	Group D (O:9)	Group C (O:6,7,8)	Group B (O:4,5)
Latex 2	Group A (O:2)	Group E (O:3,10,15) or group G (O:13,22,23)	Vi antigen

[a] Grey-brown aggregates over a cleared background indicate a nonspecific reaction.

in WCT-Shigella reagents) coated with antibodies specific to *Salmonella* or *Shigella* serogroups. When an antigen is recognized by its corresponding antibodies coating the latex particles, latex particles of a given color will agglutinate, with a contrasting change in the color of the background. Positive controls (killed *Salmonella* or *Shigella* cells of a given serogroup) for each color of latex particles were supplied with the test kits. The kits were provided with disposable suspension tubes, sampling sticks, sample dispensers, and reaction cards.

The tests were performed according to the manufacturer's directions. Bacterial cultures must be checked for smoothness in a saline control. One or two suspected *Salmonella* or *Shigella* smooth colonies from the culture plate were picked up on the flat end of a sampling stick and thoroughly emulsified in 200 μl of saline. A drop of this suspension was added to a drop of each reagent on the disposable reaction card and mixed with a sampling stick. The card was rotated on a flat-bed rotator at 150 rpm for 2 min. Reactions and changes in color were observed without removing the card. The results were visually interpreted according to the manufacturer's specifications. For WCT-Salmonella, the following reactions were observed: (i) red, green, or blue latex aggregates indicated a positive reaction (Table 1); (ii) grey-brown aggregates over a cleared background suggested a nonspecific reaction; and (iii) no aggregates, with the smooth grey-brown appearance remaining unchanged throughout the test, indicated a negative reaction (lack of group A, B, C, D, E, or G antigen or Vi antigen). As recommended by the manufacturer, bacterial suspensions of Vi-positive strains (red agglutination with reagent 2) had to be boiled for 10 min at 100°C in order to test for the presence of somatic antigen O:9 (group D; green agglutination with reagent 1). For WCT-Shigella, the following reactions were observed: (i) red or blue latex aggregates indicated a positive reaction; (ii) purple aggregates over a cleared background suggested a nonspecific reaction; and (iii) no aggregates, with the smooth purple appearance remaining unchanged throughout the test, indicated a negative reaction (lack of *S. dysenteriae* serotypes 1 to 12, *S. flexneri* serotypes 1 to 6 and variants X and Y, *S. boydii* serotypes 1 to 15, and *S. sonnei* phases 1 and 2). In positive reactions, blue aggregates with reagent 1 indicated *S. flexneri*, red aggregates with reagent 1 indicated *S. sonnei*, blue aggregates with reagent 2 indicated *S. boydii*, and red aggregates with reagent 2 indicated *S. dysenteriae*.

Of 193 presumptive *Salmonella* isolates tested with WCT-Salmonella, 182 were independently identified as *Salmonella* isolates. The 11 remaining strains were identified as *Hafnia alvei* (2 strains), *Citrobacter freundii* (2 strains), *Klebsiella pneumoniae* (1 strain), H_2S-positive *Escherichia coli* (1 strain), and *Shigella* spp. (5 strains). These non-*Salmonella* isolates did not give any visible agglutination in the test. Of these 182 confirmed *Salmonella* isolates, 179 gave unequivocal colored agglutination (i.e., blue, red, or green). Thus, they were identified to the level of the *Salmonella* serogroups detectable by the test. Thirty-nine distinct serotypes belonging to six somatic serogroups (A, B, C, D, E, and G) were identified. The distribution of serogroups and serotypes was as follows: serogroup A (2 isolates), 1 serotype; serogroup B (75 isolates), 14 serotypes; serogroup C1 (35 isolates), 7 serotypes; serogroup C2-C3 (28 isolates), 6 serotypes; serogroup D1 (32 isolates), 5 serotypes; serogroup E1 (4 isolates), 3 serotypes; serogroup E4 (1 isolate), 1 serotype; and serogroup G (2 isolates), 2 serotypes. Five isolates contained Vi antigen (red aggregates with latex reagent 2). Two isolates immediately gave a green agglutination with reagent 1 (i.e., somatic antigen O:9, serogroup D). After heating, all Vi-positive strains were positive for serogroup D. All were identified as *Salmonella* serotype Typhi. When agglutinations with WCT-Salmonella were found to be unequivocal, the correlation with the serogroup obtained by reference method was 100%. A confirmed *Salmonella* isolate failing to show any agglutination belonged to a serogroup not included in the test (serogroup F, serotype Veneziana). Two *Salmonella* isolates demonstrated purple aggregates when mixed with both reagents. Thus, the test was noninterpretable for those isolates.

Of 103 presumptive *Shigella* isolates tested with WCT-Shigella, 100 were independently identified as *Shigella* isolates. The remaining three strains, identified as *Providencia rustigianii* (one strain), *Acinetobacter baumannii* (one strain), and *Escherichia coli* (one strain), did not give any visible agglutination with latex reagents 1 and 2. Among the 100 confirmed *Shigella* isolates, all but two gave unequivocal reactions (i.e., blue or red aggregates on a cleared background) with the test. Two strains did not give visible aggregates. For one of these strains (identified as *S. dysenteriae* serotype 2), our results were confirmed by Wellcome Laboratories. The other strain, identified as *S. flexneri* serotype 4, was sent to Wellcome Laboratories in Beckenham, England, where it was found to be weakly positive with reagents from one batch (weak blue agglutination with reagent 1) but negative with reagents from another batch. The 98 *Shigella isolates* that gave unambiguous reactions with the test were correctly serogrouped. Their identifications were as follows: *S. dysenteriae* (serogroup A), 20 strains of serotypes 1 to 4, 6 to 8, and 12; *S. flexneri* (serogroup B), 37 strains of serotypes 1 to 4 and 6; *S. boydii* (serogroup C), 23 strains of serotypes 1, 2, 4, 5, 8, 9, 13, and 14; and *S. sonnei* (serogroup D), 18 strains. As noted by the manufacturers, the use of a flat-bed rotator was found essential, since manual stirring done at the early stage of this study was found unsatisfactory.

Most *Salmonella* isolates from human specimens belong to serogroups A, B, C, D, E, and G (4, 9). In our study, 162 of 163 confirmed *Salmonella* isolates of human origin were of serogroups included in the test kit. WCT-Salmonella allowed serogrouping of most *Salmonella* strains isolated from humans. Of 182 confirmed *Salmonella* isolates, 179 (98.4%) were correctly serogrouped and one (0.5%) belonged to a serogroup not included in the test kit. The two *Salmonella* isolates that gave a noninterpretable reaction (purple aggregates with reagents 1 and 2) were agglutinated by using a serum directed against fimbrial antigens (Fim antiserum). Rarely, cultures of *Salmonella* possess fimbriae which make

the bacteria O hypoagglutinable or even O nonagglutinable (8). Heating for 2.5 h at 100°C destroys Fim antigens (proteins) and restores O agglutinability. Indeed, heated suspensions tested again with the kits gave a blue agglutination with latex reagent 2 (and thus could be identified as belonging to serogroup E or G). This procedure was not described by the manufacturer. None of the 11 isolates belonging to genera other than *Salmonella* gave visible agglutination with the two latex reagents of the test (thus, the specificity was 100%). The sensitivity of WCT-Shigella when tested on pure cultures was very high: 98 of 100 (98%) *Shigella* isolates studied were serogrouped with the test. The specificity was 100%. The three non-*Shigella* isolates sent to the laboratory as presumptive *Shigella* isolates failed to react in the test. A recently published clinical evaluation of these tests (3) yielded similar percentages for sensitivity and specificity.

We found testing with these two latex kits, WCT-Salmonella and WCT-Shigella, easy to perform, accurate, and easy to interpret when pure cultures were tested. These test kits, which reduce the number of necessary reagents, can simplify the serogroup determination of *Salmonella* and *Shigella* strains.

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