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Evaluation of Commercial Antisera for *Shigella* Serogrouping

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***Shigella* serogrouping antisera from six companies (Becton Dickinson, Denka, Difco, Murex, Roach, and Sanofi-Pasteur) intended for the slide agglutination test and those of the Wellcolex Colour *Shigella* latex agglutination test were evaluated to identify quality products for *Shigella* identification. Forty-six reference *Shigella* strains (one for each serotype and species), 50 clinical strains (21 *S. flexneri*, 21 *S. sonnei*, 4 *S. dysenteriae*, 4 *S. boydii*) representing the most prevalent species and serotypes encountered in Quebec, and 9 non-*Shigella* strains were tested according to the manufacturers' instructions. A 3+ reaction (≥75% agglutination) was considered positive for the slide agglutination tests. Sensitivity varied from 47% (Roach) to 94% (Difco). For the 105 strains tested, accuracy ranged from 53% (Roach) to 91% (Wellcolex). Specificity varied from 97 to 100% for group A antisera, from 96 to 100% for group B antisera, from 88 to 100% for group C antisera, and from 95 to 99% for group D antisera. The costs of reagents required to test one strain varied from $3.50 to $13.20 (in Canadian dollars). In conclusion, Roach reagents proved to be unsatisfactory for *Shigella* serogrouping. Among those from the remaining companies, the Denka, Difco, and Wellcolex reagents met a performance standard of 90% accuracy.**

Shigella species are primarily responsible for gastrointestinal tract infections ranging from bacillary dysentery to less acute infections and are spread worldwide (1, 9–11, 13, 14). They share common characteristics with members of the genus *Escherichia*, from which they cannot be differentiated by DNA-DNA hybridization. According to modern taxonomy, subdivision of the genus into species is irrelevant. Nonetheless, medical and epidemiological interests justify maintaining the genus with its four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*, also known as groups A, B, C, and D, respectively. These are subdivided into serotypes, which are given numerical designations (5–7, 13).

Laboratory diagnosis relies on the isolation of the organism from feces and its identification by means of cultural and biochemical characteristics. Serological identification is mandatory because of its close relatedness to *Escherichia coli*. It requires, for a start, that species be identified (serogrouping); this is followed by serotype determination (serotyping). Serogrouping is generally carried out in clinical laboratories, while serotyping is performed in central or reference laboratories (5, 6, 13).

Serogrouping is based on the detection of somatic O antigens by an agglutination technique with polyclonal antisera specific to each of the four *Shigella* species. Unfortunately, there is no international consensus on the choice of *Shigella* strains for the production of the grouping antisera (6). These antisera are commercially available from various manufacturers, but the sensitivities and specificities of these products remain to be determined. A study conducted by the U.S. Centers for Disease Control in 1988 (4) showed important variations between commercial antisera manufactured in the United States. That report gave limited information on the products available in Canada and did not evaluate the Wellcolex Colour *Shigella* latex agglutination test. In that test, colored latex beads are coated with antibodies specific to each *Shigella* species, and identification depends on the color of the agglutination. Two recent studies (2, 12) indicate that this product is sensitive and highly specific for the identification of *Shigella* isolates.

In today's clinical laboratory, rapid or computerized identification systems are frequently used, and the number of conventional biochemical tests is often reduced to a minimum. This phenomenon combined with the nonspecific biochemical profile of *Shigella* species and the lack of criteria for discriminating between *Shigella* species stresses the importance of serological identification of *Shigella* species. Therefore, we decided to evaluate all commercially available products in Canada used for serogrouping of *Shigella* species in order to identify quality antisera which could aid clinical laboratories in their identification of *Shigella* species.

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MATERIALS AND METHODS

Bacterial strains. A total of 96 *Shigella* strains were included in the study (*S. dysenteriae*, 17; *S. flexneri*, 35; *S. boydii*, 22; *S. sonnei*, 22). The 46 reference strains (one per species and serotype) represent the panel of strains used at the Laboratoire de Santé Publique du Québec (LSPQ), Ste-Anne-de-Bellevue, Québec, Canada, for assessment of the quality of commercially purchased antisera for *Shigella* grouping and typing. The 50 clinical strains (*S. flexneri*, 21; *S. sonnei*, 21; *S. boydii*, 4; *S. dysenteriae*, 4) were chosen according to the prevalence of the *Shigella* species and serotypes encountered in the province of Québec. Of the 3,907 strains identified at LSPQ between 1968 and 1992, 1,849 (47%) were *S. sonnei*, 1,847 (47%) were *S. flexneri*, 110 (3%) were *S. dysenteriae*, and 101 (3%) were *S. boydii.*

Nine non-*Shigella* strains were also included in the study either because they were known to share common antigens with the genus *Shigella* or because they could be mistaken for *Shigella* species on initial isolation. They included one

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strain each of *Hafnia alvei*, *Plesiomonas shigelloides*, *Providencia alcalifaciens*, and *Yersinia enterocolitica* serotype O3 and five strains of *E. coli* (inactive strain and strains O114:H32, O157:H7, O157:H19, and ATCC 13706).

Before testing, the identification of all cultures was verified by LSPQ and was confirmed by the National Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada, with in-house antisera. The strains were then coded and maintained on a slope of egg medium at room temperature until they were needed.

Commercial grouping antisera. The *Shigella* grouping antisera from seven companies were evaluated. Six of these products were intended for use with the slide agglutination test. They were BBL *Shigella* Grouping Antisera (BBL; Becton Dickinson Microbiological Systems, Cockeysville, Md.), Denka Seiken *Shigella* Antisera (DEN; Denka Seiken Co. Ltd., Tokyo, Japan), Bacto-*Shigella* Polyvalent Antisera (DIF; Difco Laboratories, Detroit, Mich.), Murex *Shigella* Agglutinating Sera (MUR; Murex Diagnostics Ltd., Dartford, England), Roach *Shigella* Grouping Antisera (ROA; Roach Laboratories Inc., Loganville, Ga.), and Pasteur *Shigella* Agglutinating Sera (SAN; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). The seventh product tested, Wellcolex Colour *Shigella* (WCS; Murex Diagnostics Ltd., Dartford, England), was a latex agglutination test. One lot of each product was tested. All reagents were maintained according to the manufacturers' instructions and, if required, were reconstituted or diluted at the beginning of the study. The reagents needed for the study were kindly supplied by the manufacturers. The lot numbers of the antisera tested and the *Shigella* serotypes recognized by their agglutinins are listed in Table 1.

Testing procedure. Prior to testing, each strain was grown on a sheep blood agar plate, incubated overnight at 35°C, and subsequently checked for autoagglutination in saline. Growth from the 18-h agar plate was used to carry out agglutination tests according to the manufacturers' instructions. For the products from three companies (BBL, DEN, ROA) which required the preparation of a bacterial suspension, a unique dense antigen suspension was prepared by scraping growth from the plate and emulsifying it in 1 ml of sterile saline (0.85% NaCl), a volume sufficient to test all antisera from the three companies. For a given strain, the 41 commercial antisera (Table 1) were tested on the same day. Testing was done by two experienced technicians. In cases in which no agglutination was observed with any of the commercial antisera, the strain was tested for the presence of heat-labile inhibitory substances as recommended by Ewing (5). In brief, the strain was emulsified in saline, heated at 100°C for 60 min, cooled, and centrifuged, and the tests were repeated with the resulting pellet. In order to measure intralot reproducibility and product stability, testing of all strains was repeated after a delay of approximately 8 weeks.

Interpretation of agglutination tests. For the slide agglutination tests, the agglutination between a strain and a given antiserum was observed against a black background. The slide was tilted back and forth for 1 min, and the degree of agglutination was scored from 4+ to negative according to the criteria of the Centers for Disease Control (4, 16). Only clear and strong reactions of ≥3+ (at least 75% of the organisms agglutinated) were considered positive. This positivity criterion (in use at LSPQ) was submitted to all companies prior to the study. Results of the latex agglutination test (WCS) were interpreted as instructed by the manufacturer.

Analysis of results. The sensitivity, specificity, accuracy, and intralot reproducibility of the grouping antisera from each company were determined by standard methods (8). The "gold standard" was the confirmed identification of the strains by the Laboratory Centre for Disease Control with in-house antisera. For the present study, sensitivity was expressed as the percentage of *Shigella* strains detected solely by the homologous antiserum. Specificity was expressed as the percentage of heterologous *Shigella* and non-*Shigella* strains for which no false-positive reaction was observed. The practicality of each product was determined from information gathered from the two technicians performing the tests and from the characteristics of the products. To estimate the precision of the sensitivity, specificity, and accuracy values calculated for the commercial grouping antisera tested, 95% confidence intervals for proportions were determined by using TRUE EPISTAT (Epistat Services, Richardson, Tex.) software (9a).

TABLE 1. Commercial *Shigella* grouping antisera tested

Company	Grouping antisera (lot no.)	Homologous strains
BBL	A (K4GKHK)	*S. dysenteriae* 1–7
	B (A5GKQL)	*S. flexneri* 1–6, X, Y
	C (B5GKRW)	*S. boydii* 1–7
	D (B5GKRY)	*S. sonnei* I, II
DEN	A (02303)	*S. dysenteriae* 1–7
	A1 (36309)	*S. dysenteriae* 8–12
	B (90309)	*S. flexneri* 1–6, X, Y
	C (98309)	*S. boydii* 1–7
	C1 (85309)	*S. boydii* 8–10
	C2 (81309)	*S. boydii* 11–15
	C3 (26306)	*S. boydii* 16–18
	D (04309)	*S. sonnei* I, II
DIF	A (20292)	*S. dysenteriae* 1–7
	A1 (21105)	*S. dysenteriae* 8–10
	B (21384)	*S. flexneri* 1–6
	C (22281)	*S. boydii* 1–7
	C1 (18037)	*S. boydii* 8–11
	C2 (12488)	*S. boydii* 12–15
	D (20165)	*S. sonnei* I, II
MUR	A (K810310)	*S. dysenteriae* 3–10
	B (K763810)	*S. flexneri* 1–6, X, Y
	C1 (K321340)	*S. boydii* 1–6
	C2 (K812910)	*S. boydii* 7–11
	C3 (K813010)	*S. boydii* 12–15
	D (K844910)	*S. sonnei* I, II
ROA	A (2300-17)	*S. dysenteriae* 1–7
	A1 (2346-03)	*S. dysenteriae* 8–10
	B (2301-10)	*S. flexneri* 1–6
	C (2302-09)	*S. boydii* 1–7
	C1 (2344-03)	*S. boydii* 8–11
	C2 (2345-05)	*S. boydii* 12–15
	D (2303-20)	*S. sonnei* I, II
SAN	A1 (2K019Y)	*S. dysenteriae* 1, 3–6
	A2 (2K020Y)	*S. dysenteriae* 2, 7, 8
	B (2J013Y)	*S. flexneri* 1–6, X, Y
	C1 (2K019Y)	*S. boydii* 1–4
	C2 (2K019Y)	*S. boydii* 8, 10, 14
	C3 (2K020Y)	*S. boydii* 5, 7, 9, 11, 15
	D (2H013Y)	*S. sonnei* I, II
WCS	Reagent 1 (K764720)	
	Red beads	*S. sonnei* I, II
	Blue beads	*S. flexneri* 1–6, X, Y
	Reagent 2 (K764720)	
	Red beads	*S. dysenteriae* 1–12
	Blue beads	*S. boydii* 1–15

RESULTS

S. dysenteriae. The antigenic profile of group A antisera varied with the different products and did not include all serotypes. The number of homologous *S. dysenteriae* strains identified by the group A reagents is given in Table 2. The identification of strain type 8 was incorrect with MUR since polyvalent antisera A and C3 both reacted with the strain.

None of the commercial group A antisera detected strain type 13 as expected, but the strain was still checked for the presence of heat-labile inhibitory substances by the test procedure. After heating at 100°C, false-positive reactions were observed with BBL, DEN, DIF, and MUR reagent B and with DEN reagents A, A1, and D. Two other false-positive reactions occurred: MUR C3 antiserum reacted with two strains of *S. dysenteriae* type 2, to which it does not possess agglutinins.

S. flexneri. DEN, DIF, MUR, and SAN group B antisera gave positive reactions with the 35 strains tested, the WCS reagent identified 34 strains, the BBL reagent identified 31 strains, and the ROA reagent identified 24 strains (Table 3). Five strains were incorrectly identified. Although a positive reaction was observed with DEN and MUR group B antisera, DEN reagent C and MUR reagents C1 and C2 reacted with one strain each of types 3b, 4a, and 4b, and the MUR C2 reagent reacted with one additional strain each of types 3b and 4a.

According to the package inserts, DIF and ROA grouping antisera B were the only reagents which were not prepared

TABLE 2. *S. dysenteriae* strains for which a clear positive reaction was observed with the commercial group A antisera tested

Serotype (no. of strains tested)	No. of strains[a]						
	BBL	DEN	DIF	MUR	ROA	SAN	WCS
1 (2)	2	2	2	0	1	2	2
2 (4)	4	4	4	0	2	4	4
3 (1)	1	1	1	1	0	1	1
4 (1)	1	0	1	1	0	1	1
5 (1)	1	1	1	1	0	1	1
6 (1)	1	1	1	0	0	1	1
7 (1)	1	1	1	1	0	1	1
8 (1)	0	1	1	1	1	1	0
9 (1)	0	1	1	1	1	0	1
10 (1)	0	1	1	1	0	0	1
11 (1)	0	1	0	0	0	0	1
12 (1)	0	1	0	0	0	0	1
13 (1)	0	0	0	0	0	0	0

[a] Serotypes in the boxed areas represent those not covered by polyvalent antisera A, A1, and A2 or by WCS reagent 2 (red agglutinating particles); see Table 1 for details.

specifically with agglutinins to variants X and Y. Nonetheless, reference strains X and Y were detected by both polyvalent antisera. The two variants were not considered heterologous to DIF and ROA group B antisera because of the nonspecific antigenic compositions of variants X (−:7, 8) and Y (−:3, 4) which are included in the structures of other group B serotypes such as type 2a (II:3, 4) and type 5b (V:7, 8).

S. boydii. The antigenic compositions of the commercial group C antisera varied, and DEN polyvalent antisera C, C1, C2, and C3 were the only reagents to include agglutinins to the 18 *S. boydii* serotypes. The homologous *S. boydii* strains detected by the group C antisera are given in Table 4. Incorrect reactions were observed for three strains: DEN antisera A1, C1, and D, DIF antisera A and A1, and SAN antiserum A2 reacted with *S. boydii* type 15; DEN antiserum A1 reacted with *S. boydii* type 12; and SAN antiserum C3 reacted with *S. boydii* type 10. This last false-positive reaction is of interest. According to the SAN package insert, C3 antiserum includes agglutinins to *S. boydii* types 5, 7, 9, 11, and 15 but not to type 10.

TABLE 3. *S. flexneri* strains for which a clear positive reaction was observed with the commercial group B antisera tested[a]

Serotype (no. of strains tested)	No. of strains						
	BBL	DEN	DIF	MUR	ROA	SAN	WCS
1a (3)	3	3	3	3	3	3	3
1b (3)	3	3	3	3	3	3	3
2a (5)	5	5	5	5	3	5	5
2b (2)	2	2	2	2	1	2	2
3a (1)	1	1	1	1	1	1	1
3b (5)	4	5	5	5	4	5	5
3c[b] (1)	1	1	1	1	1	1	1
4a (5)	4	5	5	5	4	5	5
4b (1)	1	1	1	1	1	1	1
5 (1)	1	1	1	1	1	1	1
6 (6)	4	6	6	6	0	6	6
Variant X (1)	1	1	1	1	1	1	0
Variant Y (1)	1	1	1	1	1	1	1

[a] Antiserum B of BBL, DEN, DIF, MUR, ROA, and SAN and WCS reagent 1 (blue agglutinating particles) recognize all *S. flexneri* serotypes.

[b] Subtype 3c has been maintained in the present panel of strains, although it has been redesignated (3, 4) subtype 3b.

TABLE 4. *S. boydii* strains for which a clear positive reaction was observed with the commercial group C antisera tested

Serotype (no. of strains tested)	No. of strains[a]						
	BBL	DEN	DIF	MUR	ROA	SAN	WCS
1 (1)	1	1	1	1	0	1	1
2 (2)	1	2	2	2	0	2	2
3 (1)	1	1	1	1	0	1	1
4 (3)	1	3	3	3	1	3	3
5 (1)	1	1	1	1	0	1	1
6 (1)	1	1	1	1	0	0	1
7 (1)	1	1	1	1	0	1	1
8 (1)	0	1	1	1	1	1	1
9 (1)	0	1	1	1	1	1	1
10 (1)	0	1	1	1	1	0	1
11 (1)	0	1	1	1	1	1	1
12 (1)	0	1	1	1	1	0	0
13 (1)	0	1	1	1	1	0	1
14 (2)	0	2	2	2	1	2	2
15 (1)	0	1	1	1	1	1	1
16 (1)	0	1	0	0	0	0	0
17 (1)	0	1	0	0	0	0	0
18 (1)	0	1	0	0	0	0	0

[a] Serotypes in the boxed areas represent those not covered by polyvalent antisera C, C1, C2, and C3 or by WCS reagent 2 (blue agglutinating particles); see Table 1 for details.

Nonetheless, it reacted with type 10, while this strain was not detected by the homologous group C2 antiserum.

S. sonnei. Sensitivity was 95% (21 of 22 strains) for the DEN group D reagent, 82% (18 of 22) for DIF, 77% (17 of 22) for BBL and WCS, 73% (16 of 22) for SAN, and 18% (4 of 22) for ROA. Although MUR group D antiserum reacted with 17 strains, antiserum C3 also reacted with one of these strains, thus resulting in a sensitivity of 73% (16 of 22).

Sensitivity. Overall, the percentage of homologous *Shigella* strains correctly identified was 94% for DIF reagents, 93% for DEN reagents, 91% for SAN and WCS reagents, 85% for MUR and BBL reagents, and 47% for ROA reagents. As shown in Table 5, the denominators varied according to the number of homologous strains recognized by the commercial antisera.

Specificity. Specificity was calculated for individual grouping antisera, and according to their antigenic compositions, the number of heterologous strains varied. In addition to the previously mentioned reactions between group A, B, C, and D antisera and heterologous *Shigella* strains, cross-reactions also occurred with the non-*Shigella* strains tested. The known reaction between *P. shigelloides* (6) and group D antisera was observed with DEN, DIF, MUR, SAN, and WCS reagents. The previously reported (15) cross-reaction between *H. alvei* and *S. flexneri* was observed with BBL, DEN, DIF, MUR, and SAN group B antisera. Also, MUR group A antiserum reacted with this strain. Group D antisera for the six slide agglutination tests reacted with *E. coli* ATCC 13706. DIF group B antiserum, the MUR C1 reagent, and SAN reagents A1, A2, C3, and D reacted with *E. coli* O157:H19. No reaction was observed with the three other *E. coli* strains, *P. alcalifaciens*, or *Y. enterocolitica* serotype O3. The specificities are given in Table 5.

Accuracy. For the 105 strains tested (96 *Shigella* and 9 non-*Shigella* strains), the accuracy was 91% for the complete set of antisera from WCS, 90% for those from DEN and DIF, 89% for that from SAN, 86% for that from BBL, 81% for that from MUR, and 53% for that from ROA (Table 5).

Intralot reproducibility. All 96 *Shigella* strains were retested with each of the 41 antisera (BBL, four reagents; DEN, eight

TABLE 5. Comparative accuracies, sensitivities, and specificities of the commercial grouping antisera

Product source	Accuracy		Sensitivity		Specificity							
	Percent (no. of true positive + no. of true negative tests/ total no. of strains tested)	95% CI[a]	Percent (no. of positive homologous strains/total no. of homologous strains tested)	95% CI	Group A		Group B		Group C		Group D	
					Percent (no. of true negative strains/ total no.[b])	95% CI	Percent (no. of true negative strains/ total no.[b])	95% CI	Percent (no. of true negative strains/ total no.[b])	95% CI	Percent (no. of true negative strains/ total no.[b])	95% CI
BBL	86 (90/105)	0.78–0.92	85 (66/78)	0.75–0.92	100 (94/94)	NA[c]	97 (68/70)	0.90–1.0	100 (95/95)	NA	99 (82/83)	0.93–1.0
DEN	90 (94/105)	0.82–0.95	93 (88/95)	0.85–0.97	97 (86/89)	0.90–0.99	97 (68/70)	0.90–1.0	95 (79/83)	0.88–0.99	95 (79/83)	0.88–0.99
DIF	90 (95/105)	0.83–0.95	94 (85/90)	0.88–0.98	99 (90/91)	0.94–1.0	96 (67/70)	0.88–0.99	100 (86/86)	NA	98 (81/83)	0.92–1.0
MUR	81 (85/105)	0.72–0.88	85 (71/84)	0.75–0.91	99 (96/97)	0.94–1.0	97 (68/70)	0.90–1.0	88 (76/86)	0.80–0.94	98 (81/83)	0.92–1.0
ROA	53 (56/105)	0.43–0.63	47 (42/90)	0.36–0.57	100 (91/91)	NA	100 (70/70)	NA	100 (86/86)	NA	99 (82/83)	0.93–1.0
SAN	89 (93/105)	0.81–0.94	91 (77/85)	0.82–0.96	98 (91/93)	0.92–1.0	99 (69/70)	0.92–1.0	98 (87/89)	0.92–1.0	96 (80/83)	0.90–0.99
WCS	91 (96/105)	0.84–0.96	91 (84/92)	0.84–0.96	100 (89/89)	NA	100 (70/70)	NA	100 (86/86)	NA	99 (82/83)	0.93–1.0

[a] CI, confidence interval.
[b] Total number of non-*Shigella* and heterologous *Shigella* strains tested.
[c] NA, not applicable.

reagents; DIF, seven reagents; MUR, six reagents; ROA, seven reagents; SAN, seven reagents; WCS, two reagents) for a total of 3,936 repeat tests. With at least 8 weeks separating the original and repeat tests, the percentage of strains for which the test result remained unchanged notwithstanding the expected value was 96% (92 of 96 strains) with DEN reagents, 94% (90 of 96 strains) with DIF reagents, 92% (88 of 96 strains) with WCS reagents, 91% (87 of 97 strains) with SAN reagents, 90% (86 of 96 strains) with BBL reagents, 89% (85 of 96 strains) with MUR reagents, and 79% (76 of 96 strains) with ROA reagents. Overall, a variation rate in the test results of 1.7% (68 of 3,936 tests) was observed, with more than half ($n = 37$) of the variations occurring with *S. sonnei.*

Practical application. As reported by the two technicians, the slide agglutination tests performed from a bacterial suspension (BBL, DEN, ROA) had the following advantages: a uniform suspension, sufficient volume to carry out all of the tests, and a bacterial suspension readily available if heating at 100°C was required. However, precise instructions as to the density of the bacterial suspension lacked in the package inserts from the three companies. Interpretation of slide agglutination tests was easier with DIF and SAN reagents because colonies were directly emulsified in the antiserum without further dilution. As for the latex agglutination test (WCS), all materials required to perform the test were included in the kit. The test was easy to perform, and interpretation was less subjective.

Squeeze bottles (SAN, WCS) were easier to handle than dropper bottles, and the use of squeeze bottles saved time, but depending on the pressure exerted on the bottle, the volume of antiserum delivered varied. Particular care in squeezing the bottles had to be taken when using the SAN squeeze bottles. As for the antigenic compositions of the grouping antisera, the eight DEN reagents covered the most serotypes, while the WCS test allowed testing of the four species with only two reagents. In May 1994, the costs of the reagents (in Canadian dollars) required to test one Shigella strain were $3.50 for BBL, $4.08 for ROA, $5.12 for WCS, $7.80 for DEN, $8.30 for SAN, $9.05 for DIF, and $13.20 for MUR. For this evaluation, the size of a drop of antiserum was averaged at 50 μl. The shelf-lives of all reagents were at least 1 year.

DISCUSSION

The results of the present study indicate that ROA reagents were unsatisfactory for *Shigella* serogrouping. Accuracy and sensitivity values were significantly lower than those for the reagents from the other companies tested. Even after heating the strains at 100°C, as recommended by the company for strains that do not agglutinate, the overall sensitivity only increased from 47% (42 of 90 strains) to 72% (65 of 90 strains) (data not shown). Excessive dilution of the reagents could explain this observation and could account for the high specificity that was observed.

Variations in the performance of the reagents from the six remaining companies were observed. These may be due to a combination of the following factors: the panel of strains tested, underrepresentation of certain serotypes, subjectivity of interpretation for the slide agglutination tests, and lack of standardization of the commercial reagents.

Although representative strains of all serotypes of the four species of *Shigella* were tested, certain serotypes were, unfortunately, underrepresented because of low prevalence rates coupled with the short-term (2-year) conservation policy of clinical strains in application at that time. Furthermore, since these serotypes are also rarely isolated in other parts of the

world (1, 9, 13), it may have proven to be difficult to obtain them from another source.

In the present study, the overall specificity was not evaluated because the number of *Shigella* strains heterologous to a company's complete set of antisera was too small (e.g., one strain for DEN), and the nine non-*Shigella* strains tested were chosen specifically to verify known cross-reactions with *Shigella* antisera. Furthermore, increasing the number of non-*Shigella* strains tested would have been costly. Instead, the specificity of individual grouping antisera was measured, therefore achieving a sufficient number of heterologous *Shigella* strains.

Reactions with heterologous strains may be attributable in part to poor absorption or nonabsorption of the antisera with strains known to share common antigens. For example, absorption of group A antisera with *S. boydii* type 15 as well as absorption of group C antisera covering serotypes 12 to 15 with *S. dysenteriae*, types 2 and 8 are recommended by Ewing (5) and Ewing and Lindberg (6). Cross-reactions with these organisms were observed with DEN, DIF, MUR, and SAN reagents. With the non-*Shigella* strains, the false-positive agglutinations observed stress the importance of performing serogrouping only on those strains that have a biochemical profile compatible with *Shigella* species.

To evaluate the results for the reagents from the remaining companies, we had to take into account the variations in the antigenic compositions of the polyvalent antisera, which resulted in different denominators when sensitivities and specificities were calculated. The estimated values of sensitivity, specificity, and accuracy of the commercial reagents varied, but the confidence intervals that were calculated failed to show a difference in the performance of the reagents.

When selecting a product, a laboratory should examine the performance of the reagents, but the stabilities of the reagents and cost are also factors that will be taken into account. Intralot reproducibility was satisfactory for most reagents. The costs of the reagents required to test one strain varied from $3.50 to $13.20 when a complete set of antisera was purchased. Products with seven or eight reagents are therefore more expensive but cover more serotypes. To reduce the cost of analysis, a clinical laboratory could purchase only group B and D antisera, since *S. flexneri* and *S. sonnei* are the most prevalent *Shigella* species worldwide, and could send those strains for which a final identification of *Shigella* cannot be achieved to a reference laboratory. Testing with group B and D antisera in a first step is the standard procedure in many settings.

In conclusion, the aim of the present study was to identify quality products for *Shigella* serogrouping in the clinical laboratory. According to our results, the only unsatisfactory reagents were those of ROA. If a conservative performance standard of 90% accuracy was applied to help discriminate among the remaining companies, the reagents of DEN, DIF, and WCS would meet this criterion. Also, since shigellosis is a reportable disease in many countries, commercial reagents should be standardized and should include the best antigenic coverage to optimize *Shigella* identification.

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